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# INVESTIGATION OF THE PHYSIOLOGICAL GENETICS OF HAIR AND SKIN COLOR IN THE GUINEA PIG BY MEANS OF THE DOPA REACTION[1]

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## INTRODUCTION

KNOWLEDGE of the processes by which gene replacements bring about character differences is still meager. Although the coat color of mammals was one of the first characters studied in the field of physiological genetics little has been discovered about the effects of gene substitutions on the pigmentation process. That these genic effects are suitable for further investigation, however, is suggested by the following facts which indicate that pigment formation in mammalian hair is comparatively simple from both a genetic and a physiological point of view. Color differences seem to be due largely to a few genes with major effects. Phenotypes are not greatly changed by environmental differences within the laboratory. Pigment appears when the hair follicles are first formed and is produced continuously throughout life. The persistence of mutant spots and the autonomy of transplants indicate that many genetic differences in color are determined locally. The character itself is simple, being merely an intracellular substance, melanin. The quantity and quality of this substance can be estimated visually. Since melanin is relatively stable it can be extracted. The amount of melanin in the extract can be determined gravimetrically, titrimetrically or colorimetrically. The essential feature in melanogenesis is believed to be an oxidation reaction controlled by enzymes.

Among mammals the guinea pig is particularly favorable for study because WRIGHT (1916, 1917, 1925, 1927) has presented a genetic analysis which is more extensive than that available for any other mammal. In itself this analysis has led to conclusions which impose definite limitations on any interpretation as to the processes involved in color production and the points in these processes at which the color genes have their primary effects. Through the work of RUSSELL (1939) and HEIDENTHAL (in press), who obtained measurements of the amounts of melanin produced by most of the color combinations studied by WRIGHT, the restrictions on theory

[1] An expansion of a dissertation submitted to the faculty of the Division of Biological Sciences of the University of Chicago in candidacy for the degree of doctor of philosophy. The author is grateful to PROFESSOR SEWALL WRIGHT for suggesting this problem, for provision of material and for helpful advice during the course of the work. The investigation was aided by a grant to the University of Chicago from the Rockefeller Foundation.

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have been made more quantitative than was possible from visual grading of hair color. The next step is to relate this information to a direct biochemical study of the various color combinations. This paper is presented as a contribution to such a study.

The term "melanin" is used to describe what is probably a group of related substances with similar properties. The chemical constitution of these is not known, but the nature of their formation in plants and animals has been demonstrated. At the beginning of the century it was shown that certain plants and animals possess oxidizing enzymes capable of producing melanins from melanogens (colorless organic cyclic complexes such as tyrosine). Since then similar results have been obtained from many plant and animal species and several authors have written extensive reviews on the subject. The general conditions necessary for the formation of melanin have been reviewed by SCHMALFUSS and WERNER (1926) and by MULZER and SCHMALFUSS (1933). It is generally accepted that the production of melanin in mammals resembles that in other forms, at least to the extent of involving an oxidizing enzyme and a melanogen.

By treating the skin of mammals with a standard concentration of a suitable melanogen solution, under controlled conditions, various workers have shown that differences between certain color varieties may be due simply to differences in enzyme content or activity. KRÖNING (1930) has used this method on guinea pigs. Since any theory as to the action of color genes is bound to consider facts of this nature it seemed desirable to make a more extensive investigation in this direction, with particular reference to the gene combinations studied by WRIGHT.

## METHODS

### *Choice of method*

In investigating the role played by enzymes in pigmentation in mammals several melanogens and ways of treating the skin have been employed. It was decided to choose from among these one relatively simple, sensitive and reliable method and to concentrate on this, using as many different genotypes as possible, rather than to examine a few genotypes with a multiplicity of biochemical tests. The results of earlier workers and the author's own findings regarding the merits of various methods are presented in this section.

*Melanogens.* Of the melanogens available only those two which have been used extensively, tyrosine and dopa (l. 3,4-dioxyphenylalanine), will be discussed here. For a fuller treatment see BLOCH (1927) and MULZER and SCHMALFUSS (1933).

Numerous workers have tried to obtain a reaction to tyrosine by pigment-forming cells in frozen sections, but the results have always been

negative. On the other hand, some authors claim that extracts from certain pigmented types will darken tyrosine while extracts from unpigmented animals will not. It is to be noted, however, that even this method has proved unreliable and that, in general, reactions were obtained only with difficulty, after prolonged time or by the addition of other substances. ONSLOW (1915) was apparently satisfied with the results obtained by the addition of hydrogen peroxide to extracts of rabbit and mouse skins tested with tyrosine. PUGH (1933), working with black rabbits, claims darkening of tyrosine, after 36 hours, in only two out of a large series of experiments and questions the validity of the conclusions which ONSLOW reached from his experiments. CHARLES (1938) also criticizes ONSLOW'S work and reports a tyrosine reaction in two out of three experiments with black mice.

The claims of earlier workers, that frozen sections will not react to tyrosine, were confirmed, at least for conditions under which the same tissue will react to dopa. In the following experiment both tyrosine alone and tyrosine plus dopa (the addition of dopa, as a possible priming agent for the tyrosine reaction, was suggested to me by DR CHARLES) were tested against the appropriate controls:

Tyrosine stock solution: 0.400 gms in 1000 cc.
Dopa stock solution: 1.000 gms in 1000 cc.
The following solutions were made up:
A. 25 cc tyrosine stock plus 0.5 cc dopa stock.
B. 25 cc tyrosine stock plus 0.5 cc distilled water.
C. 25 cc distilled water plus 0.5 cc dopa stock.
Frozen sections from a black-eyed red guinea pig were tested in the following mixtures at pH 7.4 and at 38° C:
1. 10 cc A plus 10 cc buffer.
2. 10 cc B plus 10 cc buffer.
3. 10 cc C plus 10 cc buffer.
4. 10 cc dopa stock plus 10 cc buffer.
5. 10 cc distilled water plus 10 cc buffer.
Sections were removed and examined after three hours and after six hours.

The following results were obtained. Tyrosine alone (2) and its control (5) showed no reaction; tyrosine plus dopa (1) showed a very weak reaction which was, however, the same as that obtained with the same concentration of dopa alone (3); dopa alone in higher concentration (4) showed a strong reaction. The experiment was repeated and gave the same result.

Tyrosine was also tested in the presence of hydrogen peroxide in a concentration of one part of peroxide in 200 parts of the buffered mixture. Frozen sections gave no reaction in this mixture while other sections from the same piece of tissue gave a strong reaction with dopa.

It is, of course, possible that a reaction of frozen sections to tyrosine would occur under other conditions, but for the present purpose it is

sufficient to know that pigment-forming cells act more readily on dopa than on tyrosine.

A review of the conditions under which the melanoblasts of mammalian skin will react to dopa is given by BLOCH (1927). According to BLOCH the reaction occurs only when pigment is being formed and the degree of reaction is proportional to the pigment-forming activity. On the basis of this and other evidence BLOCH suggested that cells which have the capacity to produce melanin possess an oxidase ("dopa-oxidase") which is specific to dopa. BLOCH's results have been substantiated by many workers and it is generally accepted that the reaction is enzymic, but the specificity attributed by BLOCH to dopa-oxidase has met with severe criticism, a short summary of which is given by FRANKE (1934). This criticism is based on the contention that the ease with which the reaction occurs may depend on the ease with which dopa is known to oxidize rather than on the specificity of the enzyme present. At the present time there is not sufficient evidence to determine whether the dopa and tyrosine reactions of mammalian melanoblasts are due to the same enzyme, to different enzymes or even partly to the same enzyme and partly to different enzymes. The demonstration by RAPER (1928, 1932) that dopa is an intermediate product in the artificial formation of melanin from tyrosine is, however, of interest in this connection. The fact remains that dopa, whatever its role, if any, in normal pigmentation in mammals, serves as a sensitive indicator of some phase of melanin-forming activity or capacity. It has been used by many investigators on diverse problems for over two decades and no other melanogen tested on mammals has given a stronger reaction. MULZER and SCHMALFUSS (1931) claimed that oxytyramine gave as marked a reaction as dopa in frozen sections of the ear of a black guinea pig. Since they used borax in their buffer mixture, and since in the present work it is shown that borax has a marked inhibiting effect on the dopa reaction, their conclusion cannot be accepted until the work has been repeated with a suitable buffer.

On the basis of the above findings dopa was selected as the melanogen for this investigation.

*Treating the skin.* In treating the skin with melanogen two methods, already mentioned above, have been used extensively: (a) An extract is made either by grinding the skin or by freezing it and then breaking it up with pestle and mortar. With the addition of water, followed by pressing, centrifuging, filtering or similar treatment, a more or less clear liquid is obtained to which the melanogen may be added. (b) Fresh or fixed skin is sectioned on the freezing microtome (the procedures of paraffin and celloidin embedding destroy the desired reaction) and the sections immersed in a solution of the chromogen.

Two other methods have been described. BECKER, PRAVER and THATCHER (1935) found that, given sufficient time, a solution of dopa will penetrate fresh tissue. Since sectioning follows the reaction paraffin embedding can be used. The other method employs a compress. As described by SCHULMANN and KITCHEVATZ (1926) this consists of several layers of filter paper saturated in dopa solution and enclosed within a small oiled-silk envelope which has an opening cut in one side. This is applied for from five to ten hours to the living animal on an area of the skin from which the superficial layers of the epidermis have been removed by scraping.

It seems to the author that use of the extract method has the following disadvantages. Tyrosine has given unreliable results and if dopa is used there is the difficulty that dopa reacts not only with the melanoblasts, but also, as cited by BLOCH (1927), with polymorphonuclear leucocytes, both in pigmented and unpigmented skin. Presumably it was this difficulty which was encountered by CHARLES (1938) and other workers who failed to obtain a clear-cut difference in dopa reaction of extracts from pigmented and unpigmented animals. Melanoblasts occur principally in the hair bulbs and in the basal layer of the epidermis. Even if an extract free from leucocytes could be obtained there is the further objection that, in a single extract, no distinction could be made between the reaction of the basal layer of the epidermis and that of the hair bulbs, reactions which, as reported in this paper, are often quite different.

The method worked out by BECKER and his collaborators, and described above, was designed to give thin sections for histological study of the basal layer of the epidermis. For comparing reactions in hair bulbs it was found to be not as suitable as the frozen section technique.

The compress method is of no use in measuring the reaction of hair bulbs. Even as a measure of the basal layer reaction it would seem to have two serious objections: first, the difficulty of scraping deep enough to obtain a reaction and yet avoid bleeding, and second, the difficulty of standardizing the scraping to give good comparisons.

The frozen section method was, therefore, chosen for this work.

### *Details of method*

With the choice of the dopa reaction in frozen sections as the method of attack, an attempt was made to find the most favorable conditions for demonstrating any differences in reaction between the different genotypes. Since WRIGHT'S work has been concerned mostly with coat color the hair-bulb reaction was given primary attention. The techniques of other workers were compared and controversial points examined experimentally. The following factors were considered.

*1. Age.* STEINER-WOURLISCH (1925) claims to have shown that, in the mouse, pigment formation, and with it the dopa reaction, exhibit a rhythmic course during the growth of the animal. BOYD (1932) has compared adult sheep with lambs not more than three days old. She concludes that with age the production of dopa-oxidase becomes restricted to the fiber bulbs (which apparently react as strongly as ever) and can no longer be demonstrated in any great amount in the basal layer and follicle sheath. Preliminary experiments were conducted to discover whether or not the dopa reaction of hair bulbs in the guinea pig varies to any extent with age. With red animals examined at 15 different ages between a 44-day embryo and a 593-day adult there was very little difference in reaction. Since the coat color of some genotypes changes with increasing age, however, animals have, whenever possible, been tested before they were two months old.

Age in the growth cycle of individual hairs should not be confused with age of animal. Hairs in or near the club stage do not react with dopa. In a few cases a test had to be rejected because all hairs in the sample of skin sectioned were in this unfavorable stage of development. According to DAWSON (1930) the percentage of growing hairs in the guinea pig is greatest in March–April and October, and smallest in December and August, but the generality of this conclusion is questionable, for the author did not, as far as can be judged, use more than a few animals and the ages of these were not recorded. Examination of material used in the present work showed no simple seasonal influence.

*2. Plucking.* SCHULTZ (1925) and KRÖNING (1930) plucked their animals 10–14 days before the skin was tested, in order, so they claim, to obtain follicles in the same stage of growth in all experiments. The present author's observation was that plucking did not produce this result. Furthermore, in obtaining a set of data that can be compared with WRIGHT's grades plucking is undesirable because certain genotypes change color after plucking. Plucking was, therefore, avoided.

Since hairs are not uniform in color along their length it is probable that the dopa reaction varies to some extent with stage of development of follicles. Apart from avoiding hairs approaching the club stage this possible source of variation was controlled only by observing large numbers of follicles.

*3. Fixation.* Many workers have tried both fresh and formalin-fixed skin and have preferred the latter because it permits neater sections. The time of fixation recommended varies from one to five hours in five percent formalin. BLOCH states that material which has been in this fixative for three days can still be used. Experiments by the present author showed that after two days in five percent formalin the reaction of the hair bulbs

was apparently as strong as after two hours; after four days the reaction was weak; after eight days it had ceased to occur. In a comparison of fresh and fixed material no great difference in the rate of reaction could be discerned, but the fixed material seemed to show a reaction a little earlier than the unfixed. Two hours fixation in five percent formalin was adopted.

*4. Embedding.* BLOCH embedded in agar agar solution. Good sections can, however, be obtained without embedding and so this step has been avoided.

*5. Thickness of sections.* In comparing the hair-bulb reaction of different genotypes under the microscope it seemed best to use either the whole bulb or a thin section through the center. Sections of skin cut thick enough (200 microns and above) to show whole hair bulbs were found to be unsatisfactory. Thinner (30 micron) sections were, therefore, used and comparisons limited to those bulbs which showed at least a portion of the central papilla.

*6. Purity of solutions.* BLOCH purified the water used in his work by repeated distillation, and he maintained that all trace of carbon dioxide must be removed before the solutions are made up. PERCIVAL and STEWART (1930) recommend distilling successively with potassium permanganate, phosphoric acid, sodium carbonate, and finally alone. LAIDLAW and BLACKBERG (1932) claim that such precautions are unnecessary; they simply distil tap water once with potassium permanganate and take no steps against carbon dioxide. Distilling ordinary laboratory distilled water once with potassium permanganate has given consistent results in the work reported here. No precautions were taken against carbon dioxide since it was found that even the addition of sodium carbonate to sufficiently buffered dopa did not affect the reaction.

*7. Buffers.* As has been mentioned before, MULZER and SCHMALFUSS (1931) used a mixture containing borax. In experimenting with a wide range of hydrogen ion concentration it was found, in the present work, that a borax-phosphate mixture, when compared with the mixture of primary and secondary phosphates used by most authors, has a marked inhibiting effect on both the spontaneous oxidation of dopa and the reaction in frozen sections. This is in accord with the previously observed rule, quoted by MICHAELIS (1930), that borax reacts with substances containing two or more hydroxyl groups. In trying higher values of pH than can be obtained with phosphate mixtures a mixture of barbital and hydrochloric acid (MICHAELIS 1930) was used. Where their ranges overlapped, phosphate and barbital mixtures gave similar results.

Except in the preliminary experiments described above the phosphate mixture was used exclusively. The concentration of the mixture is given

in the summary of this section. Tests showed that this concentration was adequate to maintain the required pH.

*8. Concentration of dopa.* Concentrations of dopa varying from one part in 500 parts of the buffered mixture to one part in 2000 have been recommended by different authors. In preliminary experiments the weakest of these concentrations appeared adequate and was used for the rest of this work.

*9. pH.* BLOCH, and most workers, used a pH of from 7.3 to 7.4. Some authors, namely FRANKE, state that this is the optimum pH for the enzyme. This is probably a misunderstanding of BLOCH, who said that the reaction may proceed faster in more alkaline solution, but that a pH higher than 7.4 is undesirable because of the increased non-enzymic oxidation of dopa and the consequent deposition of the melanin so formed on the sections immersed in the solution. In experimenting on this point frozen sections were tested at pH values ranging from 5.6 to 9.8, using the phosphate and barbital buffers already mentioned. The results indicated an optimum just above pH 8. However, BLOCH's claim that a lower pH gives better differentiation between melanoblasts and their surroundings was substantiated. A pH of 7.4 has been used throughout.

*10. Temperature.* Most, if not all, workers have used either room temperature or blood temperature. The reaction proceeds faster at blood temperature and is, therefore, less likely to be affected by disturbing influences from the outside. MULZER and SCHMALFUSS (1933) state that pigmentation reactions in general have their optimum between 20°C and 60°C. 40°C was chosen for this work.

*11. Changing the solution during the reaction.* If more than a trace of formalin is carried over into the dopa solution it is liable to inhibit the reaction. This seems to be due to a reaction between formalin and dopa. In test-tube experiments on the spontaneous darkening of buffered dopa, in the concentration used in this work, it was found that if formalin is present in the mixture in a concentration of 0.05 percent the solution will turn orange instead of producing a dark melanin precipitate. Even a concentration of 0.005 percent is not without visible effect.

LAIDLAW and BLACKBERG (1932) recommend replacing the original solution with fresh during the reaction. This has the advantage of removing any formalin that may have come in and also prevents the accumulation of an excessive amount of the melanin formed spontaneously by the solution. After a few experiments it was decided to change the solution at half an hour and also at an hour and a half after the sections were first immersed.

*12. Time.* With the above conditions the reaction is first clearly visible after about an hour and a half, and the general darkening of the sections

begins to obscure the melanoblast reaction after about six hours. The comparisons in this work were made on sections treated for three hours.

*Summary*

Since the above treatment of method has involved considerable discussion the actual procedures adopted are gathered together in the following paragraph.

The animal was anaesthetized with ether and a small piece of skin removed from the mid-dorsal line in the lumbar region. This was fixed in five percent formalin for two hours, rinsed for a few seconds in distilled water and cut into 30 micron sections on the freezing microtome. The sections were cut at right angles to the surface of the skin and in the plane of the hair shafts. Forty to fifty sections were placed in a solution made up of 10 cc of a 1 in 1000 solution of dopa, plus 8 cc of a 2M/15 solution of secondary sodium phosphate, plus 2 cc of a 2M/15 solution of primary potassium phosphate. (This concentration of buffer gives a pH of 7.4.) Control sections were immersed in a similar mixture with 10 cc of distilled water in the place of the dopa solution. These mixtures were contained in crystallizing dishes 5 cm in diameter and 3 cm in depth. The water used for the solutions was obtained by redistilling ordinary laboratory distilled water once with potassium permanganate. The dopa solution was always made up less than 24 hours before use and mixed with the buffer only immediately before the sections were cut. Experimental and control solutions were kept at 40°C and the experimental solution was replaced with fresh at ½ hour and 1½ hours after the sections were first immersed. The replacing solutions were kept at 40°C and dopa and buffer mixed just before use. Sections were removed after three hours, rinsed in distilled water, passed through alcohols and xylol and mounted in balsam.

MATERIAL

The animals used came from an extensive series of experiments conducted by DR. SEWALL WRIGHT for the purpose of producing animals of as great a variety of known genotypes, with respect to color, as possible. The genotypes were determined by DR. WRIGHT on the basis of the genotype of the parents, the animal's own color and, in certain cases, breeding tests.

The colors of the animals used here are referred to usually only by genotype. In all tables each genotype is indicated by those genes in which it differs from the genetic formula $S-E-a\,a\,C-F-P-B-$ (symbols according to WRIGHT). For convenience in description the genotype represented by the above formula, in which is present the wild-type gene of every series of alleles except the $A-$ (agouti) series, will be referred to as "type."

### *Hair color*

The colors of the various genotypes have been discussed extensively by WRIGHT (1927), but for convenience in comparing the dopa reaction with natural pigmentation table 5 has been prepared from his data. This table shows the average grades of coat color of the genotypes studied here and also of certain other genotypes. The inclusion of more than one genotype within a single column indicates that these genotypes have the same grades of coat color. The range of colors possible within each albino (*C* −, $c^k$ $c^k$, etc.) series is given at the top of the columns. In grading, WRIGHT used two series of skins corresponding with the two apparently qualitatively different kinds of pigment which, in this paper, are denoted as sepia and yellow. Black, sepia, brown and pale sepia colors appear sufficiently alike in quality to be graded against a single series of standard skins ranging from grade 1 to grade 21. The remaining colors, red, yellow and cream, were graded against a series ranging from 1 to 13. In table 5, *e e*, *e e p p* and *e e b b* combinations are all homozygous for *F*. WRIGHT'S grades for *e e F f*, etc., combinations (not shown in table 5) were slightly less than for the corresponding *e e F F*, etc., combinations. Since the difference is so small animals used in the present work were not tested to determine whether they were *F F* or *F f*.

Red, yellow, cream, and similar animals showing "sootiness" (which usually develops with age) were avoided in the dopa work.

## RESULTS

The method chosen has produced remarkably uniform results within each genotype. For example, including preliminary experiments 23 black-eyed red animals have been examined and of these five have been examined twice. Of these 28 tests not one failed to give a marked reaction. In a total of 13 white animals none gave a reaction. There is evidence which indicates that the variation remaining within each genotype is due more to variation in color of animals and stage of development of follicles than to the method.

### *Grading*

Grading of both hair-bulb and basal-layer reactions was made as objective as possible by preventing the genotype of each animal from being known while the sections were being graded.

*Hair bulbs.* After comparison of experimental and control sections the material was divided into two groups.

The first group contains genotypes which showed so little pigment in hair bulbs of the control sections that it could not have contributed, measurably, to the grade of pigment shown in the experimental. This

TABLE 1

*Distributions and means of the grades of dopa reaction in the hair bulbs.*

| | *pp* | | | | | | | *ee* | | | | | | | *eeepp* | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4 | 5 | MEAN | 0 | 1 | 2 | 3 | 4 | 5 | MEAN | 0 | 1 | 2 | 3 | 4 | 5 | MEAN |
| | | | | | | 48 | 5.00 | | | | | 5 | 41 | 4.89 | | | | 1 | 16 | | 3.94 |
| | | | | | | 24 | 5.00 | | | | 73 | 3 | | 3.04 | | | | | 4 | | 4.00 |
| *C* — | | | | | | 13 | 5.00 | | | | | 3 | | 4.00 | | | 2 | 78 | | | 2.98 |
| | | | | | | 81 | 5.00 | | | | 1 | 11 | 4 | 4.19 | | | | 4 | 89 | | 3.96 |
| | | | | | | | | | | | | 21 | 21 | 4.50 | | | | | 58 | 7 | 4.11 |
| | | | | | | | | | | | | 52 | 8 | 4.13 | | | | | | | |
| | | | 8 | 23 | 1 | | 2.78 | | 2 | 12 | 3 | | | 2.06 | | 15 | 22 | | | | 1.59 |
| $c^k c^k$ | | | | 1 | 14 | 4 | 4.16 | | 116 | | | | | 1.00 | | 16 | 18 | 1 | | | 1.57 |
| | | | 17 | 30 | 4 | | 2.75 | | 2 | 1 | | | | 1.33 | | 3 | 35 | 5 | | | 2.05 |
| | | | 1 | 25 | 4 | | 3.10 | | 4 | 8 | 2 | | | 1.86 | | | | | | | |
| $c^k c^d$ | | | 7 | 2 | | | 2.22 | | | | | | | | | 18 | 11 | | | | 1.38 |
| | | | 6 | 2 | | | 2.25 | | 32 | 11 | | | | 1.26 | 2 | 32 | | | | | 0.94 |
| $c^k c^r$ | | 5 | 3 | | | | 1.38 | | 59 | | | | | 1.00 | | | | | | | |
| | | 3 | 50 | 1 | | | 1.96 | | | | | | | | | | | | | | |
| | | 4 | 23 | 1 | | | 1.89 | | | | | | | | | | | | | | |
| $c^k c^a$ | | | 75 | 16 | | | 2.18 | | | | | | | | 3 | 6 | | | | | 0.67 |
| | 10 | | 29 | | | | 1.74 | | | | | | | | | | | | | | |
| | | | 39 | 83 | 5 | | 2.73 | | 3 | 20 | 8 | | | 2.16 | | 2 | 5 | 4 | 1 | | 2.33 |
| $c^d c^d$ | | | | | | | | | 4 | 41 | 3 | | | 1.98 | | | 40 | 14 | | | 2.26 |
| | | | | | | | | | | 2 | 16 | 4 | | 3.09 | | | | | | | |
| | | | | | | | | | | 19 | 5 | | | 2.21 | | | | | | | |
| | | | 3 | 4 | | | 2.57 | | 10 | 8 | | | | 1.44 | | 28 | 5 | | | | 1.15 |
| $c^d c^r$ | | 28 | 13 | | | | 1.32 | | | | | | | | | | | | | | |
| | | 30 | 6 | | | | 1.17 | | | | | | | | | | | | | | |
| | | 11 | 54 | | | | 1.83 | | | | | | | | | | | | | | |
| $c^d c^a$ | | 18 | 1 | | | | 1.05 | | 13 | 2 | | | | 1.13 | | 29 | 5 | 4 | | | 1.34 |
| | | 13 | | | | | 1.00 | | | | | | | | | | | | | | |
| | 51 | | | | | | 0.00 | 9 | | | | | | 0.00 | 27 | | | | | | 0.00 |
| $c^r c^r$ | 16 | | | | | | 0.00 | | | | | | | | | | | | | | |
| | 25 | | | | | | 0.00 | | | | | | | | | | | | | | |
| | 3 | | | | | | 0.00 | 53 | | | | | | 0.00 | | | | | | | |
| $c^r c^a$ | 8 | | | | | | 0.00 | | | | | | | | | | | | | | |
| | 4 | | | | | | 0.00 | | | | | | | | | | | | | | |

TABLE 1 (*Continued*)

| | *fpp* | | | | | | | *eeff* | | | | | | | *eeffpp* | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4 | 5 | MEAN | 0 | 1 | 2 | 3 | 4 | 5 | MEAN | 0 | 1 | 2 | 3 | 4 | 5 | MEAN |
| | | | 8 | 8 | | | 2.50 | | 3 | 8 | 12 | | | 2.39 | | | 2 | 2 | | | 2.50 |
| *C* — | | 1 | 13 | 6 | | | 2.25 | | | | | | | | | | 2 | 7 | | | 2.78 |
| | | | | | | | | | | | | | | | | | 3 | 30 | 56 | | 3.60 |
| $c^d c^d$ | | | | | | | | 2 | 22 | | | | | 0.92 | 6 | 8 | | | | | 0.57 |
| | | | | | | | | 13 | 23 | | | | | 0.64 | | | | | | | |

TABLE 1 (*Continued*)

| | *p p b b* | | | | | | | *e e b b* | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4 | 5 | MEAN | 0 | 1 | 2 | 3 | 4 | 5 | MEAN |
| *C* – | | | | | 17 | 14 | 4.45 | | | | | | | |
| $c^k c^k$ | | | | | | | | | | 2 | | | | 2.00 |
| | | | | | | | | | 14 | | | | | 1.00 |

group is comprised of animals with red, yellow or cream natural pigment and the pale sepia (*p p*) and pale brown (*p p b b*) combinations. In the experimental sections of the above group every suitable bulb was graded under the microscope (magnification 100×) by comparison with a series of standard bulbs. The standards chosen represented six grades ranging from unpigmented (grade 0) to dark sepia or black (grade 5). As earlier workers have observed, the product of the reaction is always sepia in quality, even in red animals, and one series of standards was sufficient. Table 1 contains only the genotypes, mentioned above, in which the amount of natural pigment was negligible and in which the dopa reaction could, therefore, be graded directly. Within each genotype box in table 1 each row represents the reaction in a single animal. The figures show both the number of hair bulbs falling within each grade and, calculated from these, the mean reaction for each animal. Figure 1A shows a control section from that genotype in table 1 which has most natural pigment. That this amount of pigment may be disregarded when grading the amount of dopa-melanin formed is shown by figure 1B, an experimental section from the same animal. Two genotypes, $c^r c^r$ *p p* and $c^r c^a$ *p p*, in table 1, recorded as giving no reaction, showed pigment in the experimental sections, but, as far as could be judged, no more than was present in the control sections. In these two cases it is, of course, possible that a very weak reaction would not be detected.

Results obtained from the second group of material, in which the amount of natural pigment was not negligible, are shown in table 2. Since the grades given were made by mentally subtracting the amount of pigment in the control from that in the experimental they cannot be regarded as more than approximate estimates of the actual reactions. Even when the control is extremely dark it is still possible to get an estimate from the fact that when a strong reaction occurs the bulb presents a solid black appearance, while the pigment in the control appears arranged in discrete granules or clumps of granules. An albino and the white area of a tri-color animal are listed in table 2 for convenience of tabulation although the control sections of these contained no pigment and, therefore, gave no difficulty in determining a lack of reaction.

*Basal layer of the epidermis.* Here the natural pigment appeared sepia

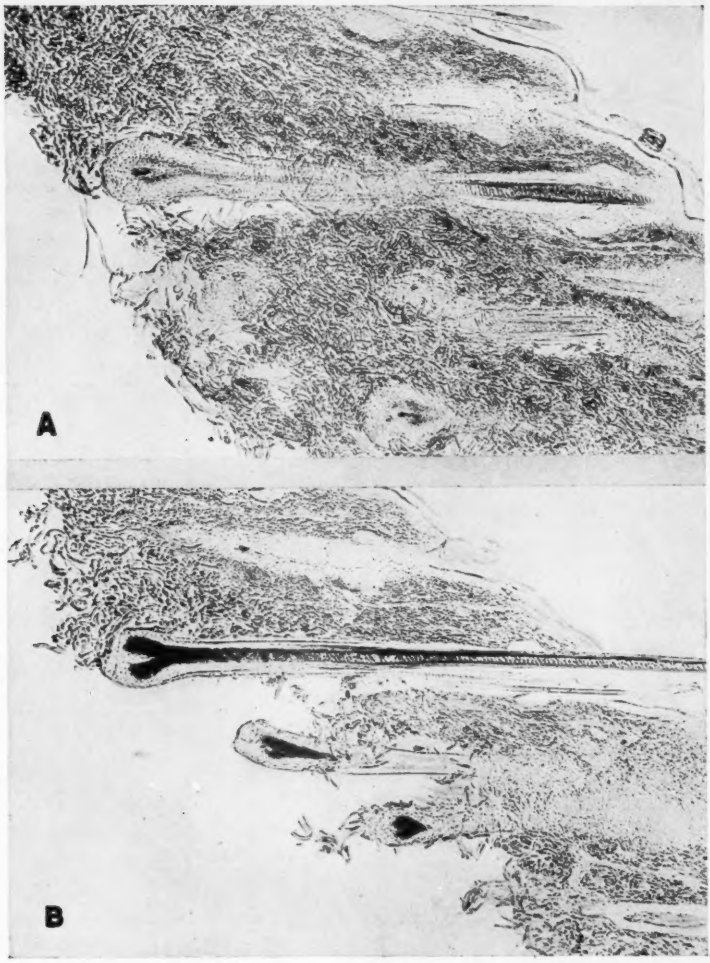


FIGURE 1.—A. Photomicrograph of a control section from an animal of genotype *p p*. (Magnification 60 diam.) 2. B. Photomicrograph of an experimental section, showing dopa reaction, from the same piece of skin that provided the control section shown in A. (Magnification 60 diam.)

or black even in animals with red hair color. Reactions were estimated by comparison with their controls. The results are given in table 3. Each row within each genotype box represents a single animal. There are two entries for each animal. The left entry indicates the amount of pigment in the control as judged by comparison with standards classified as: unpigmented

TABLE 2

*Estimated dopa reaction in the hair bulbs of those genotypes in which accurate grading of the reaction is prevented by the presence of much natural pigment.*

| | TYPE | *f f* | *b b* | *A* — | *s s* $c^p$ $c^p$ | *s s* $c^p$ $c^p$ *b b* |
|---|---|---|---|---|---|---|
| *C* — | 5 (2 animals: 39 and 165 hair bulbs) | 2–4 (1 animal: 38 hair bulbs) | | 5* (1 animal: 144 hair bulbs) | Black area 5 (1 animal: 41 hair bulbs) / White area† 0 (1 animal: 19 hair bulbs) | Brown area 4–5 (1 animal: 155 hair bulbs) |
| $c^d$ $c^d$ | 2–4 (1 animal: 40 hair bulbs) | | | | | |
| $c^d$ $c^r$ | 2–4 (1 animal: 87 hair bulbs) | | | | | |
| $c^r$ $c^r$ | 0–2 (1 animal: 13 hair bulbs) | | 0–2 (1 animal: 59 hair bulbs) | | | |
| $c^a$ $c^a$ | White area† 0 (1 animal: 20 hair bulbs) | | | | | |

* Control showed 20 bulbs in the sepia-producing phase and 3 bulbs in the yellow-producing phase.

† Grading accurate in these cases.

(O), pale (P), medium (M) and dark (D). A trace of pigment occurring in two cases is indicated by O+. The right entry gives an estimate of the reaction graded as: no difference between experimental and control (O), a weak reaction (W), a strong reaction (S).

### *Skin color*

Since extensive studies on the pigmentation of the epidermis in the different color varieties of the guinea pig are not available the following survey was made from the control sections listed in table 3.

It has been stated already that the pigment appears sepia or black, even in red animals. In most pigmented animals dendritic cells containing

pigment can be clearly seen in the basal layer and, in some animals, most, if not all, of the pigment present is confined to these cells.

TABLE 3

*Natural pigmentation and dopa reaction in the epidermis.*

| | TYPE | *pp* | *ee* | *eepp* | *ff* | *ffpp* | *eeff* | *eeffpp* | *bb* | *ppbb* | *eebb* | *A* − | *ssePeP* | *ssePePbb* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | D S<br>M S | O O<br>O O<br>O W<br>O O | P S<br>P S<br>P S<br>P S<br>M S<br>M S<br>M S | O O<br>O+W<br>O O<br>O W<br>O+W | M S | O O<br>O O | P S | O O<br>O O<br>O O | | O O | | M S | Black area<br>P S<br>White area<br>O O | Brown area<br>P S |
| *Ck Ck* | | O O<br>O O<br>O O<br>O O | M O<br>P O<br>P O<br>P O<br>M O<br>P O | O O<br>O O<br>O O | | | | | | | P O<br>P O | | | |
| *Ck Cd* | | O O | | O O | | | | | | | | | | |
| *Ck Cr* | | O O<br>O O<br>O O<br>O O | P O<br>P O | O O | | | | | | | | | | |
| *Ck Ca* | | O O<br>O O | P O | O O | | | | | | | | | | |
| *Cd Cd* | D O | O O | P O<br>P O<br>P O<br>P O<br>P O | O O<br>O O | | | P O<br>P O | O O | | | | | | |
| *Cd Cr* | P O | O O<br>O O<br>O O<br>O O | P O | O O | | | | | | | | | | |
| *Cd Ca* | | O O<br>O O | P O | O O | | | | O O | | | | | | |
| *Cr Cr* | P O | O O<br>O O<br>O O | P O | O O | | | | | P O | | | | | |
| *Cr Ca* | | O O<br>O O<br>O O | P O | | | | | | | | | | | |
| *Ca Ca* | White area<br>O O | | | | | | | | | | | | | |

From table 3 it is apparent that the order of effect of the various genes on skin color is similar to that found in the eyes. WRIGHT's determinations of the effects of the various genes on eye color can be described briefly as follows. There is no effect of *s s*, *e e* or *A* −. With respect to the albino

series, combinations involving $c^k$ and $c^d$ have little or no effect, but black is reduced to dark red by $c^r\,c^r$, to a light red by $c^r\,c^a$ and to pink (no pigment) by $c^a\,c^a$. It is important to note in the above that the order differs from that of sepia in the coat. There is no effect of $f\,f$. There is extremely little pigment left in the eye in $p\,p$ (eye pink). Finally, $b\,b$ reduces black to dark red or "brown." Combinations of two or more deviations from type have the effects expected. The actual pigment present in the eye in all cases is sepia in quality. Each of the following facts concerning skin color disagrees with the sepia, or the yellow, or both kinds of pigment in the hair, while it agrees with the pigment found in the eye: (a) the sepia quality of the pigment; (b) the absence, except for an occasional trace, of pigment in all combinations with $p\,p$; (c) the presence of pigment in $e\,e\,c^r\,c^r$ and $e\,e\,c^r\,c^a$; (d) the lack of reduction when $f\,f$ is substituted for $F-$. A possible contrast with the eye is in the effect of $E-$, but, with the amount of variation observed in the natural pigmentation of the epidermis, the evidence is not sufficient to determine whether the greater amount of pigment recorded in some $E-$ as compared with the corresponding $e\,e$ combinations indicates a real difference between these combinations or not. If the difference is real it is still possible that careful histological examination of the eye would reveal parallelism even here.

### *Dopa reaction in the hair bulbs*

Table 4 was prepared from tables 1 and 2 to show the mean reaction for each genotype as calculated from the means for animals treated as units.

TABLE 4

*Mean dopa reaction of the hair bulbs in each genotype.*

| | TYPE | $pp$ | $bb$ | $ppbb$ | $ff$ | $ffpp$ | $A-$ | $ssc^pc^p$ BLACK AREA | $ssc^pc^p$ WHITE AREA | $ee$ | $eepp$ | $eebb$ | $eeff$ | $eeffpp$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $C-$ | (5) | 5.00 | (4–5)* | 4.45 | (2–4) | 2.38 | (5) | (5) | 0.00 | 4.13 | 3.80 | | 2.39 | 2.96 |
| $c^k\,c^k$ | | 3.20 | | | | | | | | 1.56 | 1.74 | 1.50 | | |
| $c^k\,c^d$ | | 2.22 | | | | | | | | | 1.38 | | | |
| $c^k\,c^r$ | | 1.87 | | | | | | | | 1.13 | 0.94 | | | |
| $c^k\,c^a$ | | 1.96 | | | | | | | | | 0.67 | | | |
| $c^d\,c^d$ | (2–4) | 2.73 | | | | | | | | 2.36 | 2.30 | | 0.78 | 0.57 |
| $c^d\,c^r$ | (2–4) | 1.72 | | | | | | | | 1.44 | 1.15 | | | |
| $c^d\,c^a$ | | 1.03 | | | | | | | | 1.13 | 1.34 | | | |
| $c^r\,c^r$ | (0–2) | (0) | (0–2) | | | | | | | 0.00 | 0.00 | | | |
| $c^r\,c^a$ | | (0) | | | | | | | | 0.00 | | | | |
| $c^a\,c^a$ | 0.00 | | | | | | | | | | | | | |

Bracketed figures are estimates of the reaction in those genotypes in which accurate grading of the reaction is prevented by the natural pigment present.

* This estimate was made from the brown area of an animal of genotype $s\,s\,c^p\,c^p\,b\,b$.

The broad features of the data will be considered first. An effect of *s* in the combination *s s* $e^p$ $e^p$ is shown by the absence of any reaction in the white area of an animal that gave a full reaction in its black area. The mixture of yellow and sepia hair bulbs in the control of the agouti (*A* −) animal tested show that the hair bulbs in the experimental animal were probably of both types also. As far as they go the results indicate no difference in reaction between the two phases of color production found in this genotype. Combinations with *e e* in general show slightly less reaction than the corresponding combinations with *E* −. The effect of the *C* − series of alleles is to give three general levels of reaction: strong with *C* −; no reaction with $c^r$ $c^r$, $c^r$ $c^a$ and $c^a$ $c^a$; and intermediate with the

TABLE 5

*Average grades of coat color as determined by* WRIGHT.

| | TYPE | *p p* | *bb* | *p p b b* | *ff* | *ff p p* | *ee* *ee p p* *ee b b* | *ee ff* *ee ff p p* |
|---|---|---|---|---|---|---|---|---|
| | | BLACK SEPIA WHITE | PALE SEPIA WHITE | BROWN WHITE | PALE BROWN WHITE | BLACK SEPIA WHITE | PALE CREAM WHITE | RED YELLOW CREAM WHITE | YELLOW PALE CREAM WHITE |
| *C* − | | 21.0 | 9.7 | 15.6 | 8.3 | 20.9 | 2.2 | 10.6 | 6.8 |
| $c^k$ $c^k$ | | 20.1 | 9.1 | 14.6 | 7.2 | 19.0 | | 7.1 | 1.2 |
| $c^k$ $c^d$ | | 19.4 | 7.6 | 15.4 | 6.0 | | | 7.2 | |
| $c^k$ $c^r$ | | 20.5 | 7.0 | 14.8 | 6.1 | | | 4.6 | |
| $c^k$ $c^a$ | | 18.5 | 5.5 | 13.6 | 5.6 | 18.0 | | 4.6 | 0.0 |
| $c^d$ $c^d$ | | 16.9 | 4.9 | 14.2 | 5.4 | 16.8 | 0.0 | 7.0 | 1.6 |
| $c^d$ $c^r$ | | 19.1 | 4.0 | 15.0 | 3.7 | 18.6 | | 4.1 | 0.2 |
| $c^d$ $c^a$ | | 14.0 | 2.6 | 12.5 | 3.2 | 13.9 | | 4.2 | 0.1 |
| $c^r$ $c^r$ | | 20.1 | 2.6 | 15.5 | 2.8 | | 0.0 | 0.0 | 0.0 |
| $c^r$ $c^a$ | | 15.5 | 0.7 | 13.1 | 1.1 | | | 0.0 | 0.0 |
| $c^a$ $c^a$ | | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | | 0.0 | 0.0 |

remaining homozygotes and heterozygotes. Combinations with *f f* show less reaction than the corresponding ones with *F* −. Substituting *p p* for *P* − seems to have little or no effect on the reaction. If there is any effect from substituting *b b* for *B* − it is only a slight reduction.

The effects of the genes may now be considered in more detail. Tests of significance of the difference between *P* − and *p p*, between *E* − and *e e*, and between *F* − and *f f* combinations were made using STUDENT'S method for paired comparisons. The genotypes which were paired differed only in the alleles under consideration. Thus, in testing the significance of the difference between *P* − and *p p* combinations, *e e C* − *P* − (4.13) was paired with *e e C* − *p p* (3.80), *e e* $c^k$ $c^k$ *P* − (1.56) with *e e* $c^k$ $c^k$ *p p* (1.74) and similarly for eight comparisons in all. This test did not give a significant difference (P = 0.89). Testing *E* − against *e e* in nine com-

parisons showed a significant difference (P=0.03). Testing $F-$ against $ff$ in five comparisons gave a significant difference (P about .004).

Turning to the $C-$ series it is of interest to determine how many significantly different levels in intensity of reaction can be distinguished between the strong reaction given by $C-$ and the absence of reaction found with $c^r\,c^r$, $c^r\,c^a$ and $c^a\,c^a$. It will be seen from table 4 that the $C-$ series has been tested extensively in three combinations with other genes, namely: $p\,p$, $e\,e$ and $e\,e\,p\,p$. With $p\,p$ the order of reduction in reaction in the intermediate combinations is: $c^k\,c^k > c^d\,c^d > c^k\,c^d > c^k\,c^a > c^k\,c^r > c^d\,c^r > c^d\,c^a$. With $e\,e$ the order is: $c^d\,c^d > c^k\,c^k > c^d\,c^r > c^k\,c^r = c^d\,c^a$. With $e\,e\,p\,p$ the order is: $c^d\,c^d > c^k\,c^k > c^k\,c^d > c^d\,c^a > c^d\,c^r > c^k\,c^r > c^k\,c^a$. If an attempt is made to divide these intermediate genotypes into two levels of reaction it will be seen that there is only one line of cleavage which is consistent for all of the three groups. This places $c^k\,c^k$, $c^k\,c^d$ and $c^d\,c^d$ at the higher level and $c^k\,c^r$, $c^k\,c^a$, $c^d\,c^r$ and $c^d\,c^a$ at the lower level. A possible interpretation of this particular cleavage will be discussed later. The means for animals in the combinations $e\,e$ and $e\,e\,p\,p$, which, as already noted, do not differ significantly, were combined and the difference between the two levels indicated above was tested by STUDENT'S method for unpaired comparisons, following the conservative procedure of calculating the standard deviation from the combined data instead of from the deviations from the separate group means. (Standard error of the difference $=\sigma_T\sqrt{1/n_1+1/n_2}$, where $\sigma_T$=standard deviation of combined population, $n_1$=number in first group, $n_2$=number in second group.) The difference between the two levels (represented by 14 and 8 animals respectively) proved significant (P about 0.006). Similar tests between each of the intermediate levels and the $C-$ level (represented by 11 animals) also proved significant (P<0.001 in each case). Thus, altogether, four significantly different levels of reactions were obtained with combinations of albino alleles in the presence of $e\,e$ and $e\,e\,p\,p$. For convenience these levels will be designated as: $C-$, $c^{k\text{ or }d}\,c^{k\text{ or }d}$, $c^{k\text{ or }d}\,c^{r\text{ or }a}$ and $c^{r\text{ or }a}\,c^{r\text{ or }a}$.

### *Dopa reaction in the basal layer of the epidermis*

In white areas of spotted animals the basal layer of the epidermis, like the hair bulbs, shows no dopa reaction. The effect of the $C-$ series agrees in direction, but the threshold is higher (above all combinations except $C-$) than that (between $c^r\,c^r$ and $c^{k\text{ or }d}\,c^{r\text{ or }a}$) in the hair bulbs. Substituting $p\,p$ for $P-$ results in complete, or almost complete, loss of the reaction, whereas in the hair bulbs the same substitution has little or no effect. Substituting $e\,e$ for $E-$, or $A-$ for $a\,a$, has no apparent effect. Combinations with $ff$, as far as can be judged, show as strong a reaction as the corresponding combinations with $F-$, a result which differs from

that found in the hair bulbs. Finally, substituting *b b* for *B*− gives no recognizable reduction in the dopa reaction.

It should be noted that the differences between the reactions in the basal layer and the reactions in the hair bulbs are not all in the same direction. The lower members of the *C*− series and *p* give more reduction in the basal layer than in the hair bulbs (*p* none at all in the latter), while *e* and *f* give reduction in the hair bulbs but none in the basal layer.

## DISCUSSION

### *Results of other workers*

It is of interest to compare the results obtained here with those reported in rabbits by SCHULTZ (1925) and in guinea pigs and rabbits by KRÖNING (1930). The three sets of results are in agreement in finding a strong reaction in "type" and no reaction in the white parts of white-spotted and albino animals. In the dark extremities of albino guinea pigs and in the intermediate combinations $c^k\ c^k$ and $c^k\ c^a$,[2] both in the presence of *E*− and *e e*,[3] KRÖNING claims that the reaction was as strong as with *C*−. KRÖNING further claims that in chinchilla rabbits the agouti-banded hairs reacted with full intensity irrespective of whether the bulbs were in the sepia-producing or the white-producing phase. SCHULTZ states that yellow rabbits gave a weak reaction. KRÖNING reports no difference in guinea pigs between black and red, nor between pale sepia and pink-eyed red, and suggests that, since comparisons with black are difficult, SCHULTZ may have been mistaken. The present results, indicating a slight reduction in *e e* combinations as compared with *E*− combinations, stand in an intermediate position. KRÖNING claims that substituting *p p* for *P*− reduces the reaction in the presence of either *E*− or *e e*. The present results agree with this in direction in so far as the basal layer is concerned, but in the hair bulbs there was, as has already been stated, no evidence of a reduction either in the combinations studied by KRÖNING or in many others. SCHULTZ found that brown rabbits reacted slightly less strongly than black (this was confirmed by KRÖNING), an effect which is in agreement with the present results as far as they go. The factor *f* has not been studied by earlier workers.

KRÖNING does not describe the method which he used for measuring the reaction, and such differences as exist between his results and those

[2] KRÖNING used the symbol $c^H$ for WRIGHT'S $c^a$, and his symbol $c^a$ probably corresponds to WRIGHT'S $c^k$. For convenience in the above discussion the symbols have been changed to match WRIGHT'S.

[3] In some cases KRÖNING used skin from the red areas of tortoise-shells, $e^t\ e^t$ (WRIGHT'S $e^p\ e^p$), instead of from self reds, *e e*, but since it is not always clear which of the two was used, only *e e* is written throughout this discussion.

reported here are seen to be differences in degree rather than direction. His final conclusions are that: (a) apart from the effect in $c^a\ c^a$ (which he does not attempt to explain) there is no unequivocal relation between dopa reaction and the albino series; (b) the quantity of dopa enzyme present depends on the *P* factor; (c) the natural chromogen is determined by the gene *E*, and the lack of chromogen in *e e* is replaceable with dopa. Some of the observations on which these hypotheses are based have, as already noted, not been confirmed by the present study. Results obtained from gene combinations not available in KRÖNING'S work have a further bearing on his conclusions and will be discussed in the next section of this paper.

### *Physiological genetic interpretations*

Since the dopa reaction leads always to a black or sepia end-product, whereas two apparently qualitatively different pigments, sepia and yellow, are produced in guinea-pig hair, it could hardly be expected that the effects of the genes on the dopa reaction would provide a complete explanation of the physiological genetics of both sepia and yellow pigment formation. From the color of the dopa reaction, and from KRÖNING'S conclusions, a parallelism of the reaction and the grades of sepia pigment was expected. It is surprising to find that the results in the hair bulbs show no such parallelism, but do indicate a close agreement of degree of reaction with grades of yellow pigment. These conclusions will now be considered in detail.

On the simplest interpretation of KRÖNING'S conclusion it was expected that the hair-bulb reaction would, throughout both *E*− and *e e* combinations, agree with the order of effect found in the sepia pigments of the coat. This has not proved to be the case. No combination with $c^r\ c^r$ has shown a definite reaction. This holds even in the presence of *E*−. For example, *C*− *b b* and $c^r\ c^r\ b\ b$ have almost identically the same average grade of coat color, yet $c^r\ c^r\ b\ b$ gave either no reaction or, at most, one very much less than that given by *C*− *b b*. Arguing further against a correspondence with the sepia pigments is the lack of effect of *p* upon the reaction and the marked effect of *f*. This is a complete reversal of the effects of these genes on the natural sepia pigments.

The reaction of the hair bulbs, in *e e* combinations at least, does, however, agree closely with the grades of yellow pigment found in these combinations. The lack of effect of *p*, and possibly *b*, and the marked effect of *f*, are paralleled in the grades of natural yellow pigment. Even more striking is the agreement in the order of reduction with the albino series. Reference has already been made to four significantly different levels of reaction, *C*−, $c^k$ or $^d\ c^k$ or $^d$, $c^k$ or $^d\ c^r$ or $^a$ and $c^r$ or $^a\ c^r$ or $^a$, found in the com-

binations *e e* and *e e p p*. These levels correspond to the natural colors, red, yellow, cream and white, found with the same four groups of albino alleles in the presence of *e e*. On the assumption on which the present work was undertaken, that the dopa reaction indicates the presence of an oxidizing enzyme system, the simplest interpretation of the above results is that gene replacements at the *C* and *F* loci alter the amount of yellow pigment produced by affecting the concentration or activity of the enzyme system present.

As far as they go, results in the hair bulbs in the presence of *E* indicate that the reaction, in its threshold and order of effect with the *C*− series, its reduction by *f* and lack of reduction by *p* much more nearly parallels the intensity of yellow in the corresponding *e e* combinations than it does the intensity of the sepia pigments actually present in the *E*− combinations tested. There is, however, an apparently significant intensifying effect of *E* over *e*. These facts, while interesting, do not, at the present time, provide a simple clue to the natural biochemical effect of gene replacement at the *E* locus or, related to this, the order of effect of the remaining genes in *E*− combinations. It appears likely that this part of the problem will not be solved by the dopa method alone.

Turning now to the epidermis, it has already been pointed out that the natural pigmentation in this location is quite different from that in the hair. It is, therefore, apparent that the final effects produced by interaction of the color genes depend upon regional factors. In the two paragraphs following it will be seen that the dopa results furnish evidence on both the physiological genetics of the epidermis and the nature of the biochemical difference in the two locations, skin and hair.

From table 3 it is evident that in the albino series the threshold for the dopa reaction is higher than that for pigment formation, the reaction occurring, in fact, only with *C*−. It may be that a weak reaction occurred with lower alleles, but was obscured by the natural pigment, while a reaction with *C*− was strong enough to be clearly visible. It has already been stated that the evidence of a difference in natural pigmentation between *E*− and *e e* combinations is not regarded as sufficient. Apart from the apparent threshold difference with the *C*− series, and a possible lack of agreement in the effect of *E*, the reaction parallels the natural pigmentation in the epidermis, and, in view of the fact that there is little or no reaction in all *p p* combinations, it cannot be said to parallel any kind of pigment in the hair. The simplest physiological interpretation is that, in the basal layer of the epidermis, the lower alleles of the *C*− series and *p* produce their effects, at least in part, by reducing the concentration or activity of the enzyme system present.

Regarding the difference between epidermis and hair bulbs, it is seen

that the different effects produced by the same gene combinations in the two regions are paralleled, in *e e* combinations at any rate, by differences in the dopa reaction. It follows, again on the simplest interpretation, that one biochemical result determined by regional factors is a difference in the enzyme systems active in the two locations.

So far the effect on the dopa reaction of the gene *s* has not been considered either in hair bulbs or epidermis. In white-spotted animals there is clearly a failure of enzyme in the white spots, but whether this means a change in the physiology of the melanoblasts or actual absence of these cells is not shown.

## SUMMARY

In an attempt to elucidate some of the processes by which color genes produce their effects the following results were obtained:

1. Natural pigmentation in the epidermis is similar to that, observed by WRIGHT, in the eye, both in its quality and in the order of effect of the genes studied.

2. The technique used in treating frozen sections of skin with a buffered solution of dopa produced adequately uniform results within each genotype.

3. The effect of gene *s* was shown by the absence of any dopa reaction in the white area of a white-spotted animal that gave a full reaction in the black area. Combinations with *e e* gave, in the hair bulbs, slightly, but significantly, less reaction than the corresponding combinations with $E-$, while there was no difference between the reactions in the basal layer of these two combinations. As far as the evidence goes there was no difference in reaction between the two phases of color production found in agouti, $A-$, and the reaction obtained agreed with that found in *a a*. As compared to a strong reaction with $C-$ the lower alleles of the albino series gave no reaction in the basal layer and reduced reaction, or no reaction, in the hair bulbs. In the hair bulbs four significantly different levels of reaction were shown: none with $c^r$ or $^a$ $c^r$ or $^a$ combinations, weak with $c^k$ or $^d$ $c^r$ or $^a$, medium with $c^k$ or $^d$ $c^k$ or $^d$ and strong with $C-$. Combinations with *f f*, as compared with $F-$, gave no difference in reaction in the basal layer, but significantly less reaction in the hair bulbs. Combinations with *p p* showed almost no reaction in the basal layer, while the hair bulbs gave as strong a reaction as those in the corresponding $P-$ combinations. If the gene *b* had any effect in the few combinations in which it was tested, it can only have been a slight reduction.

The following physiological genetic interpretations are made:

1. Gene replacements at the *C* and *F* loci alter the amount of natural yellow pigment produced in the hair by affecting the concentration or activity of the enzyme system present.

2. In the epidermis the lower alleles of the $C-$ series and $p$ produce their effects, at least in part, by reducing the concentration or activity of the enzyme system present.

3. There is a difference in the enzyme systems active in pigmentation in the two locations, skin and hair.

4. There is a failure of enzyme in the white areas of $s\ s$ animals.

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# RECOMBINATION IN SPECIES CROSSES

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## INTRODUCTION

IN THE field of mathematics there are numerous problems difficult to solve with finite numbers which become relatively simple when the concept of infinity is introduced. Similarly, though the genetics of species crosses is obscure when considered in terms of single factors, it seemed possible that it might be illuminated if one attempted to consider the *total* recombination of specific differences. The recombinations in any particular cross could be compared, as a whole, with the particular species combinations from which they arose, or the total recombination in one cross compared with that in another. Such an experiment with *Nicotiana Langsdorffii* and *N. alata* was started in 1930 at the John Innes Horticultural Institution. It was carried forward at the Missouri Botanical Garden the following year and at the Bussey Institution of Harvard University from 1931 to 1933 while the author was a staff member of the Arnold Arboretum. Grateful acknowledgment is made to these institutions and to DR. HENRY W. EDMONDS and MRS. RUTH OWNBEY for faithful and expert assistance in collecting the data and preparing it for publication. I am indebted to DR. PRISCILLA AVERY of the University of California for unpublished information in regard to chiasma frequencies. A preliminary account was presented at the Sixth Genetics Congress (ANDERSON 1932), and the chief conclusions were incorporated in a technique (ANDERSON 1936) for the study of hybrid populations. Publication *in extenso* has been deferred until the conclusions could be checked experimentally with other genera and until certain phases of the work (ANDERSON 1939) could be given a more complete statistical treatment. Though the method is simple it leads to somewhat unexpected conclusions. It furnishes new criteria for the recognition and analysis of hybridization in natural or artificial populations and should prove of practical assistance to the plant breeder.

## MATERIALS AND METHODS

The species chosen for the cross were two species of *Nicotiana* which are native to South America (AVERY 1938), *Nicotiana alata* and *N. Langsdorffii*. For such an experiment it was desirable to have morphologically dissimilar species which yield hybrids with a high degree of fertility. These conditions are met in relatively few genera. Species which are partially fertile *inter se* and yet which are morphologically as dissimilar as these two species are most exceptional. In the author's experience they are to

be found elsewhere only among the orchids and in the genera *Narcissus* and *Aquilegia*. These all present cultural difficulties which prevent their use in large scale experiments. These two species of *Nicotiana*, however, can be grown readily in greenhouses of experimental gardens and several generations can be grown in one year.

The *Nicotiana alata* used in the experiment is the clear white, large-flowered sort grown in gardens under the name of *N. affinis*. COMES, in his monograph (1899) of the genus, designated it as *N. alata* Lk. and Otto var. *grandiflora* and concluded that it was merely a cultivated form of *N. alata*. For the sake of brevity it will be referred to throughout this paper as *N. alata*. The strain used was obtained from MESSRS. SUTTONS as *N. affinis* in 1930. Ten plants were grown and were found to belong to at least five inter-fertile, intra-sterile classes, in so far as their cross-sterility relationships were concerned. Two inter-fertile plants, nos. 86-5 and 86-7, were chosen for the experiment. It should be pointed out that such strains as this one have been grown in flower gardens for many years and have been consciously and unconsciously selected during that time for size of flower, whiteness of flower, and proportionally long corolla tubes. They probably therefore have accumulated a number of modifiers for these three characters which might conceivably affect the results of the experiment.

The *N. Langsdorffii* was obtained from the Royal Botanic Gardens, Kew, England. The plants as grown at the John Innes Horticultural Institution all seemed uniform. An open-pollinated seed capsule from one plant gave rise to sixteen seedlings, all essentially similar. Nos. 55-11 and 55-13 were chosen for the experiment. *Nicotiana Langsdorffii* has also been garden-grown for many generations but since it is a botanical curiosity rather than a flower for the perennial border, the strain used in this experiment is probably not greatly different genetically from the wild species.

To guard against exceptional results due to the interaction of self-sterility alleles and to detect the presence of exceptional gene differences within the species, the crosses were made as shown in figure 1. Two individuals of each species were selected, and reciprocal intra-specific as well as inter-specific crosses were made. The cross *N. alata*×*N. Langsdorffii* is usually sterile but with persistence a number of capsules were obtained. All these crosses were made numerous times so that the parental species could be grown for comparison with each generation. The second generation was obtained in two ways, by selfing $F_1$ plants and by crossing two plants of the $F_1$ with each other. The theoretical basis for discriminating between these two types of $F_2$'s has been published elsewhere (ANDERSON 1935) and is discussed below under Selective Fertilization (page 681).

For several reasons the experiment was confined to a study of the flowers: (1) As shown by EAST (1913, 1916) in his classical work on quantitative characters, flower size is less affected by environmental variables than is the size of vegetative parts. (2) *Nicotiana alata* and *N. Langsdorffii* do not differ as strikingly in vegetative characters as they do in floral characters.

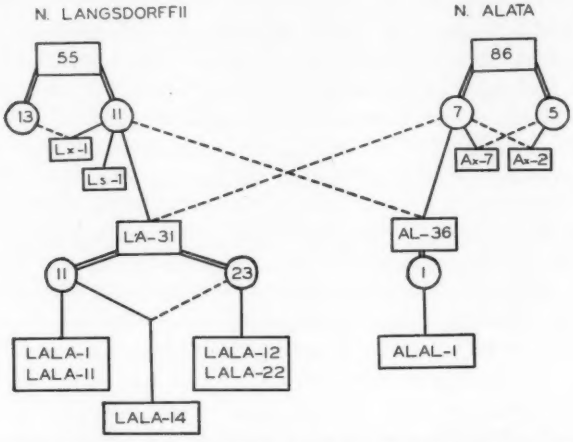


FIGURE 1.—Chart illustrating the relationships of the families used in this experiment. Rectangles represent families, circles denote individual plants.

Typical flowers of the two species are shown in figure 2 drawn to the same scale. The chief differences may be tabulated as follows:

| *Nicotiana alata* | *N. Langsdorffii* |
|---|---|
| Corolla clear white | Corolla green |
| Pollen ivory | Pollen bright blue |
| Night blooming | Day blooming |
| Corolla tube long (5.8–8.9 cm) | Corolla tube short (1.7–2.1 cm) |
| Corolla limb wide (2.5–4.0 cm) | Corolla limb narrow (0.6–0.9 cm) |
| Corolla limb deeply lobed | Corolla limb scarcely lobed |
| Corolla lobes serrate, acuminate | Corolla lobes broadly crenate |

Not all of these differences could be scored readily. Day blooming versus night blooming is affected by temperature and humidity as well as by daylight. After a number of trials this character had to be abandoned. Pollen color is affected by the age of the pollen, particularly in various hybrid combinations which have dark blue pollen in young flowers which fades in bright sunlight to a barely perceptible blue. This character was also difficult or impossible to score in plants which were sterile or semi-sterile and it was therefore abandoned. The crenation of the corolla lobes is a very interesting character but the $F_2$ and successive generations pre-

sented such variability that it was not possible to work out a simple method of scoring the shapes.

The other characters presented fewer difficulties. When large numbers of plants are grown the green-flowered and white-flowered plants are not absolutely discontinuous in the $F_2$ as had been reported in previous experiments in which fewer plants were grown (SACHS-SKALINSKA 1921; EAST 1916). The ratios of green to white suggested that a single gene was involved plus a large number of modifiers. For the purposes of this experiment the plants were scored as pure white (opening flowers pure white untinged by green), yellowish (opening flowers yellow, more or less tinged with green, some of these flowers fading to an almost pure white) and green (flowers various shades of green, not fading to a clear yellow, never appearing whitish). The length of the flower was measured in two ways: (1) The length of the tube. (2) The length of the style (and stigma) from the top of the ovary to the tip of the stigma.

The size of the corolla limb and amount of lobing were scored by measuring the length of the longest lobe and of the shortest of the two adjacent sinuses. The lobing is scored by obtaining the ratio of these two figures, "maximum lobe/adjacent sinus." This gives values of around 1 for *N. Langsdorffii* and of about 2 or 3 for *N. alata*. Aside from the fact that it introduces a ratio (or a difference if turned into logarithms) into correlation tables this proved to be an efficient and objective way of scoring lobing and has been used by other experimenters (SMITH 1937).

## PRESENTATION OF DATA

It will be remembered that the object of the investigation was to consider the actual recombination obtained in the second generation as compared with the total imaginable recombination of all the characters of both parents. For the reasons given above we shall confine our data largely to the corolla. The chief differences between the corollas of the two parental species (fig. 2) are (1) in tube length, (2) in limb width, (3) in the lobing of the limb, and (4) in the color of the corolla. There are many other slight differences, notably in the shape of the margin (irrespective of the lobing), in the pouching of the corolla throat, in pollen color, in time of blooming, etc. A study of the recombination of these four characters, however, will give us an excellent approximation to total recombination of corolla differences.

*Tube length and limb width.* The correlation diagram in figure 3 illustrates the recombination actually obtained in one $F_2$ of 118 plants. The values of the parental species and of the $F_1$ are shown for comparison. It will be seen that the values of the $F_2$ fall entirely within an ellipse running diagonally across the diagram. Although there is much variability

from plant to plant in the second generation, in so far as recombinations of these two characters are concerned, it is as a whole mostly in one direction, that is, from a combination of characters more or less like that

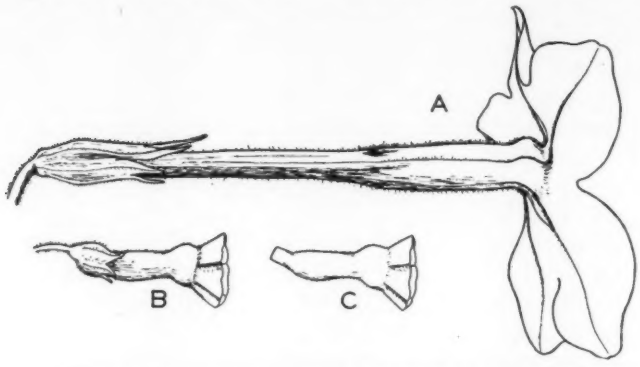


FIGURE 2.—Representative flowers of (A) *Nicotiana alata* and (B) *Nicotiana Langsdorffii;* (C) corolla of *N. Langsdorffii.*

of *N. Langsdorffii* to one more or less like that of the $F_1$ to one more or less like that of *N. alata.* There is nothing like free recombination of the two characters and the frequencies suggest that, even with an infinitely

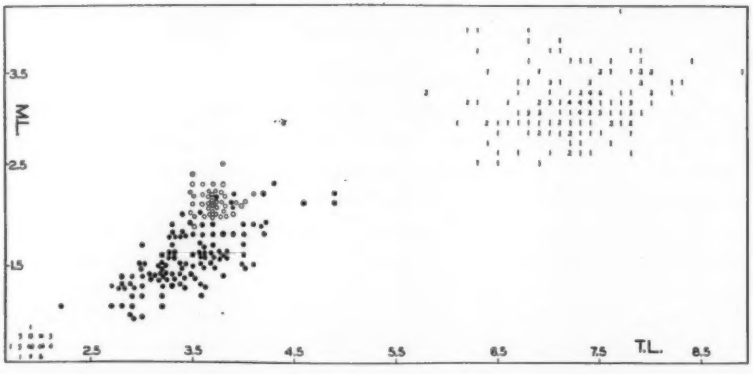


FIGURE 3.—Correlation between limb width (maximum lobe) and tube length in *Nicotiana Langsdorffii, N. alata,* one $F_1$ family of 41 plants, and one $F_2$ family of 118 plants. Open circles represent the $F_1$, solid circles the $F_2$, numbers *N. Langsdorffii* (lower left) and *N. alata* (upper right).

large $F_2$, a plant with a tube as long as that of *N. alata* and limb as short as that of *N. Langsdorffii* would not be achieved.

***Tube length and degree of lobing.*** In figure 4 are presented the data on these two characters, from the same plants which furnished the data for

figure 3. It will be seen that though there is a little more freedom of recombination than in the previous case, the recombinations are confined to a diagonal band across the correlation diagram.

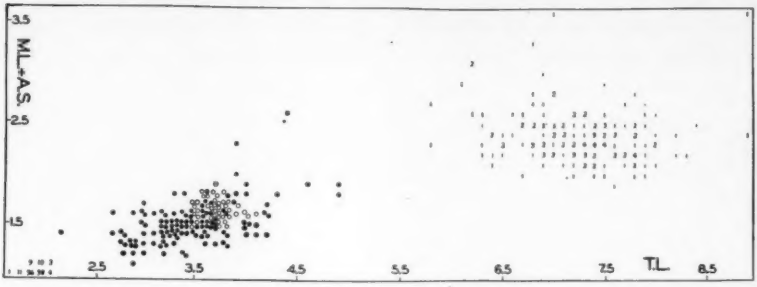


FIGURE 4.—Correlation between tube length and lobing in the plants represented in figure 3.

*Limb width and degree of lobing.* The recombinations of these two characters, for the same $F_2$, are summarized in figure 5. It will be seen that the situation is substantially the same as in the other two cases.

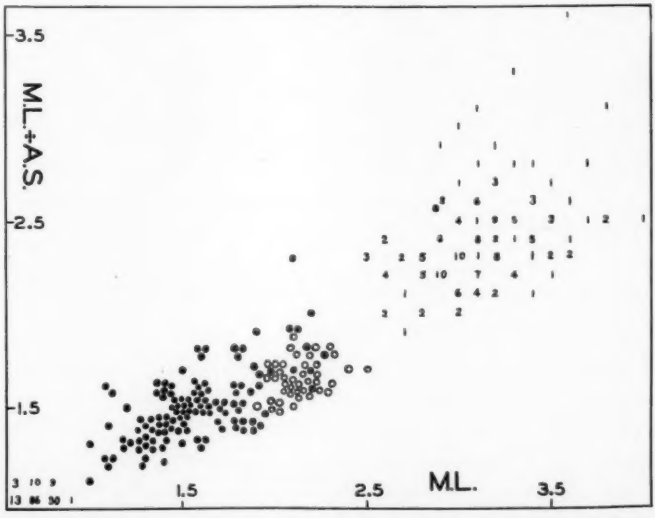


FIGURE 5.—Correlation between limb width and lobing in the plants represented in figure 3.

*Corolla color and corolla shape.* The recombination of corolla color and shape are presented graphically in figure 6. For this purpose data from a number of $F_2$ families, all grown in the same year on the same plot, have been summarized. It will be seen that there is a very strong tendency

for the flowers which are most like *N. alata* in shape to be white or whitish and for those which are shaped like *N. Langsdorffii* to be green. This tendency would appear even more clearly if a three dimensional diagram were prepared which included limb width as well as lobing and tube length. This particular phase of recombination has been studied exhaustively by SMITH, in the very similar cross between *N. alata* and *N. Sanderae*. He

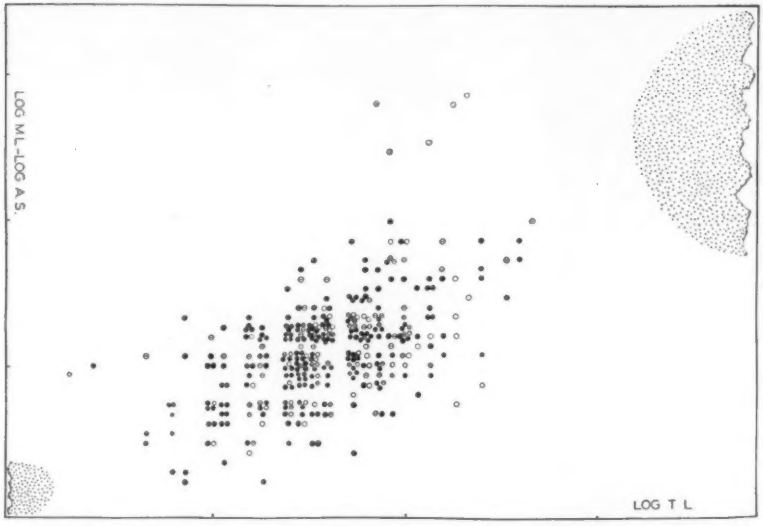


FIGURE 6.—Diagram showing the correlation between tube length, lobing of the corolla, and color of the corolla. Combined data from five $F_2$ families, representing 337 plants. Stippled areas (shown in part) represent values of *Nicotiana Langsdorffii* (lower left) and *N. alata* (upper right). Open circles denote plants with white corollas; solid circles, with green corollas; and cross-barred circles, with yellowish (intermediate) corollas. Dots somewhat grouped due to the use of two-place logarithms.

found definite evidence of linkage between corolla shape and genes affecting corolla color (SMITH 1937).

*Corolla length and style length.* The very slight recombination in these two characters was commented upon by EAST (1916) in his classical experiments. Actual data are presented in figure 7. It will be noted that in this case there is very strong correlation in these two characters in the $F_1$ as well as in the $F_2$. The significance of this fact is discussed below. For the present we need only note that, as in the case of the other flower characters, recombination in the $F_2$ is limited to a narrow ellipse running diagonally across the correlation diagram.

From the evidence submitted above it is clear that the combinations

of the $F_2$, bewildering in their variety though they may seem, are far from a complete shuffling of the traits of the two species. Just how far they fall short of being a complete assortment may be determined by generalizing the data in figures 3 to 7. If we consider the recombinations of any two characters, the actual recombinations obtained form more or less of an ellipse running diagonally across the correlation rectangle from one parental combination to the other.

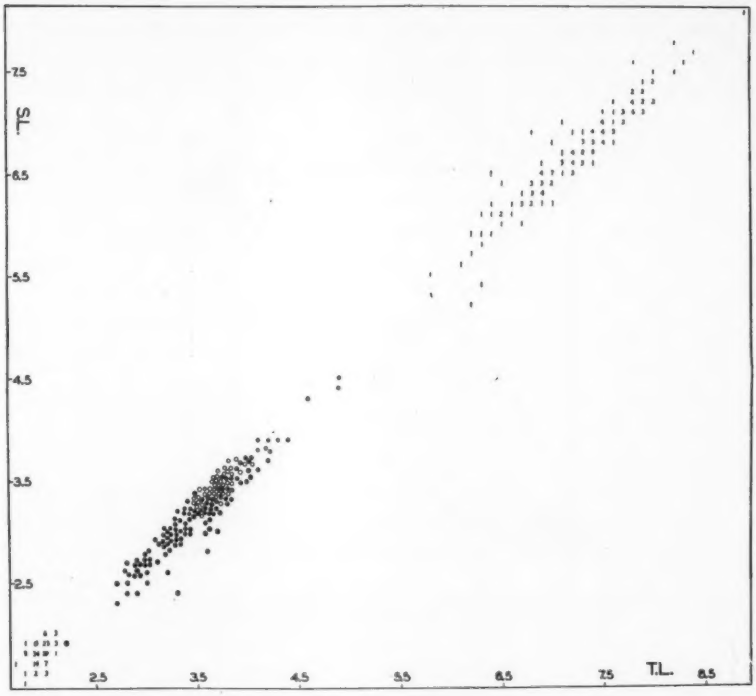


FIGURE 7.—Correlation between tube length and style length in the $F_1$ and $F_2$ represented as in figures 3, 4, and 5.

The frequencies of the correlation diagrams suggest that if larger $F_2$'s were raised the ellipses might eventually reach and just include the areas of the parental species. In other words they approach this value as a limit and even in an infinitely large $F_2$ the combinations would still fall in an ellipse whose edges (on its cross axis) would be only slightly nearer to the upper left hand and lower right hand corners than they are in the $F_2$'s reported above. As will be shown below there are also good theoretical grounds for such an hypothesis.

Had we obtained all possible recombinations of these two characters they would be distributed over the entire rectangle. The ratio of the kinds of combinations actually obtained (the frequencies are another matter) to the total combinations which might be imagined will therefore be the ratio of the area of the dark colored ellipse in figure 8 to the area of the entire square. The exact ratio in any particular example will depend upon the shape of the ellipse. It is possible, however, to consider the general case. Whatever the figure of the ellipse it tends to be symmetrical on both its axes. [Exceptions are for the most part due to the use of a scale which is warped in one way or another. The elaboration of a scale which would measure units of equal biological value (WRIGHT 1926) for *N. alata*, *N.*

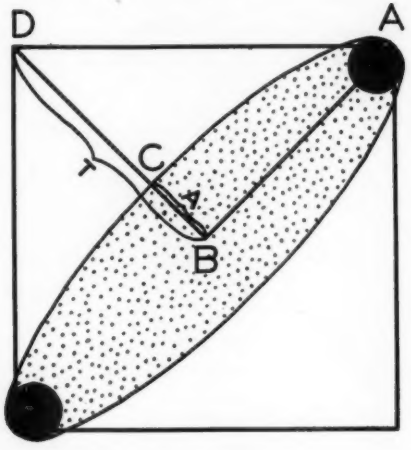


FIGURE 8.—Diagrammatic generalization of $F_2$ correlation for two multiple factor characters Solid black: areas of parental species; stippled: area of the $F_2$.

*Langsdorffii* and their recombinations is an exceedingly intricate, though rather irrelevant, problem. The unadjusted metric scale used in figures 3, 4, 5 and 7 is obviously progressively "inflated" in the direction of *N. alata*, whose total variability on a true biological scale would be only slightly greater than that of *N. Langsdorffii*. No simple transformation is very much better. A logarithmic scale is only a slight improvement (fig. 6). In studying figures 3 to 7, therefore, it should be remembered that on scales whose units were of equal biological magnitude along their whole length, the areas of *N. Langsdorffii* and *N. alata* would be approximately equal and approximately circular, and the recombination bands across the figures would be symmetrical ellipses.] We may therefore make the easier comparison of one-quarter of the ellipse to one-quarter of the rec-

tangle. In figure 8 this will be the ratio of the area ABC to ABD. Since these two areas have one dimension in common (AB), the ratio between them will be given by some function of a/t. If AC were a straight line both areas would be triangles and the actual ratio would be a/t. We may use it for the purposes of generalization, remembering that the value in any particular example will be somewhat higher or lower, depending upon whether the line AC circumscribes somewhat more area or somewhat less area than would a straight line between these points.

If we consider the recombinations of three characters our correlation surface becomes a correlation solid and the recombinations obtained fall in a spindle-shaped solid connecting two diagonal corners (fig. 11). The spindle and the rectangular solid have one dimension in common and differ by two dimensions. In each of these latter the ratio between the spindle and the outer solid is a/t. The ratio of actual recombinations to total recombinations will therefore be some function of $(a/t)^2$. The general ratio we are looking for will be a function of $(a/t)^{n-1}$, where n is the number of character differences which are being considered.

In *Nicotiana alata*×*N. Langsdorffii*, for example, if we consider only the differences in tube length, in the lobing index, in style length, and in limb width, the ratio will approach $(a/t)^{4-1}$. From an inspection of figures 3 to 7 the ratio of a to t is seen to be somewhere in the neighborhood of 1 to 4. This means that the recombinations obtained are only $(1/4)^3 = 1/64$ of the kinds of recombinations which might be obtained with free assortment. These four characters, however, represent only a few of many differences which might be considered between *N. alata* and *N. Langsdorffii*. It is therefore certain that the recombinations which we have obtained are only an insignificant fraction of the recombinations possible under free assortment.

To a non-mathematical mind this may seem too strong a statement. When the data are presented, as for the most part they necessarily must be, in terms of the recombination of two characters at a time, it takes a peculiar sort of geometric imagination to see that the proportion of actual recombinations to total recombinations becomes increasingly smaller as more characters are considered. Anyone who has examined second-generations or backcrosses of species hybrids will have been so impressed by their variability that it will be difficult for him to accept the conclusion that such a mélange is only a small fraction of total recombination. For such biologists, and as a sort of graphical summary of all the data, figures 9 and 10 have been prepared. In figure 9 are illustrated the extreme types of corollas which might be expected in the second generation if there were free recombination of tube length, limb width and lobing. In figure 10 are shown the closest approaches to these extremes, which were actually ob-

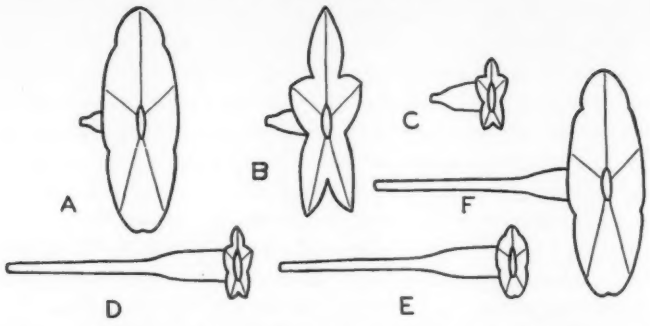


FIGURE 9.—Extreme recombinations to be expected with complete recombination of tube length, limb width and lobing of *N. alata* and *N. Langsdorffii*. Letters correspond to those used in figures 10 and 11.

tained among 347 $F_2$ plants. In figure 11 the data of figures 3 to 7 have been slightly idealized and combined into a three way correlation diagram showing the relation between total recombination for these three characters and the actual recombinations obtained in the experiment. A comparison of figures 9 and 10 with figure 11 will show that the mathematical deductions are indeed correct. The second generation extremes which at first seemed so variable become impressively uniform when compared to the imaginary recombinations of figure 9.

## DISCUSSION

The data reported above show that the recombinations of the $F_2$, however manifold they may seem, are in reality but a narrow segment of the total imaginable recombinations of the parental species. There are in other words, powerful restrictions to character recombination in the $F_2$. These restrictions come under at least four headings:

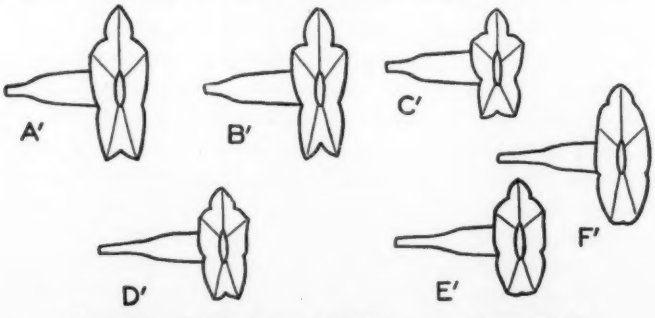


FIGURE 10.—Actual extremes obtained among 347 plants of the $F_2$. Letters correspond to those used in figures 9 and 11.

1. Gametic elimination
2. Zygotic elimination
3. Pleiotropy
4. Linkage

*Gametic elimination* is particularly important in plants because of the long and intricate gametophytic existence preceding the production of the sperm and egg. An indication of the amount of this elimination can be

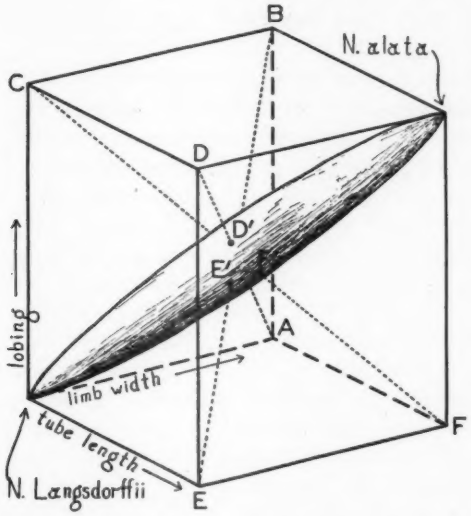


FIGURE 11.—Diagram of correlation cube for three characters, showing the relation between complete recombination (total cube) and actual recombination (central spindle). Letters mark the positions of the ideal (A to F) and actual (A′ to F′) extreme recombinations illustrated in figures 9 and 10. Three recombinations (A′, B′ and C′) are supposed to be on the far side of the solid spindle.

obtained by examining the pollen of the $F_1$ plants. AVERY has recently (1938) made a critical cytological study of the $F_1$ between *N. alata* and *N. Langsdorffii*, and reports an average of 44 percent of good pollen as judged by its morphology and staining reactions. While the bulk of the non-viable pollen must, according to her observations, be due to gross irregularities in chromosome distribution (see below, page 689, under linkage), a small percentage may well be due to the formation of gametically inviable recombinations of the two species. It might logically be expected that such combinations would be found more often among extreme recombinations than among those which are close to the original parental types.

How much recombination would be restricted by further gametic elimination during pollen germination, and fertilization we have as yet no means of knowing.

Gametic elimination also occurs due to selective fertilization by the $F_1$ pollen. Precise information is available for only one locus in the germplasm, the self-sterility alleles having been the subject of detailed investigation. Such work as that of JONES (1928), however, makes it probable that various other loci may be affected. The action of the self-sterility locus has been the subject of detailed analysis by EAST and his students (EAST 1929).

The theoretical effect of the interaction of the self-sterility alleles upon character recombination in species crosses has been discussed elsewhere (ANDERSON 1935), and will be considered here only in relation to the cross under discussion. *Nicotiana Langsdorffii* is homozygous for the self-sterility alleles $S_fS_f$. At this locus in *N. alata* there are a series of self-sterility alleles designated as $S_1$, $S_2$, $S_3$, etc. The essential feature of these self-sterility alleles is that pollen bearing any one of them will not grow rapidly enough to cause fertilization in the styles of a plant which possesses the same allele. A plant of the constitution $S_2S_4$, for instance, cannot be fertilized by either $S_2$ or $S_4$ pollen. If we designate the *alata* parent of the cross we are studying as $S_nS_m$, thereby indicating any two of the self-sterility alleles, (actually one of the alleles was identical with EAST's $S_{10}$) half of the $F_1$ plants will be of the constitution $S_fS_n$ and the other $S_fS_m$. It is possible to derive an $F_2$ from these plants in two different ways, by selfing an $F_1$ plant, or by crossing plants of these two different constitutions. The results will be as follows:

$$S_fS_n \text{ selfed} = S_fS_f + S_fS_n$$
$$S_fS_n \times S_fS_m = S_fS_f + S_fS_n + S_fS_m + S_nS_m$$

In the first (selfed $F_1$) there is selective fertilization due to the interaction of the $S_fS_n$ style and $S_n$ pollen. In the second ($F_1 \times F_1$) there is complete assortment of the four alleles. For the self-sterility locus the make-up of the $F_2$ will be three-quarters from *N. Langsdorffii* and only one-quarter from *N. alata*, instead of the normally expected half and half. The self-sterility gene, however, will not be alone, but will be accompanied by a whole segment of *alata* genes, probably a half of a chromosome or more (see below). In so far as the genes within this segment affect the morphology of the plant we may expect the first type of $F_2$ to be slightly less like *N. alata* on the average, than is the second type.

Suppose for instance, that the difference in tube length between *N. alata* and *N. Langsdorffii* were due to ten essential genes, one in each chromosome. *N. alata* would be of the genetic constitution *AABBCCDD-*

*EEFFGGHHIIJJ* and *N. Langsdorffii aabbccddeeffgghhiijj* and the $F_1$, *AaBbCcDdEeFfGgHhIiJj*. If we assume no dominance and an additive effect for each gene, then the tube of *Langsdorffii* would be K+0 units long, that of *alata* K+20 and the $F_1$ K+10 units long. The frequencies for tube lengths from K+0 to K+20 in the $F_2$ without selective fertilization will be given by the coefficients of $(a+b)^{20}$ since there are 10 pairs of genes. If one of these ten, however, is closely linked with the self-sterility allele then the frequencies of the male gametic combinations will be given by $(a+b)^9$ rather than by $(a+b)^{10}$ and the frequencies of the $F_2$ will consequently be those of $(a+b)^{19}$. These theoretical frequencies for the two types of $F_1$'s are graphed in figure 12 for comparison with actual frequencies for tube length and lobing index, obtained in these two types of $F_1$'s. Crosses with DR. EAST's homozygous stock plants made it possible to distinguish between $S_fS_n$ and $S_fS_m$ individuals. (One of the sterility alleles was identified as $S_{10}$ by its cross-incompatibilities, the other was not located among the homozygotes available for test. This reaction, however, made it possible to distinguish with certainty between $S_fS_n$ and $S_fS_{10}$ individuals among the $F_1$'s). The theoretical curves are seen to be very similar to those actually obtained. (To make the curves roughly comparable the frequencies have been graphed as percentage of total frequency). It will be seen that the curves for $(a+b)^{20}$ and $(a+b)^{19}$ when adjusted to the same total frequency differ (1) in position, (2) in shape, the theoretical curve for selective fertilization having a broader peak. The curves for tube length and lobing index show similar differences. In each case the curve for $F_1$ selfed (selective fertilization) is slightly nearer the range of *N. Langsdorffii* than is the curve for $F_1 \times F_1$. Furthermore, in each case, the $F_2$'s in which selective fertilization is taking place have a slightly higher proportion of values near the mode.

The differences between the two types of $F_2$'s are not quite as great, however, as those predicted on the basis of one gene per chromosome. If, as the work on linkage suggests [see SMITH (1937) and below pp. 684 to 691] there are two or more essential genes per chromosome then we should predict a difference of just such a magnitude between the two types of second generations as was actually observed.

*Zygotic elimination.* As to how many recombinations are eliminated after fertilization we have as yet no precise information. There are indications that such elimination can take place in the seed capsule, in aging seeds, and shortly after germination. The complexity of the problem is indicated by a comparison of $F_2$'s grown in the Bussey greenhouse and in the experimental plots out-of-doors. In the greenhouse the plants were subject to infection with mosaic disease from several interspecific hybrids of *Nicotiana* which because of their scientific interest were not destroyed in spite

of their diseased condition. Though isolated in another range of the greenhouse there was always a small percentage of infection. Out-of-doors there was almost no mosaic but in wet seasons a wilt disease was common. Since *Nicotiana alata* is fairly susceptible to mosaic though resistant to wilt while *Nicotiana Langsdorffii* is susceptible to wilt but very resistant to mosaic, zygotic elimination was quite different in the greenhouse from what it was in the field. The plants which were killed by the wilt were for the most part not yet in flower, yet from those which flowered it was evident that they closely resembled *N. Langsdorffii*. While the numbers in the greenhouse were too small to be significant the opposite tendency was evident there. No records were kept of these two $F_2$'s, which had been grown for another purpose. A precise experiment was planned for the next year but a drier season and better seed-bed sanitation practically eliminated the wilt. From these experiments, however, and from observations of natural hybrids (some of them under controlled conditions) the author is of the opinion that an $F_2$ between any two species is a very sensitive indicator of the environment. The complexion of any particular $F_2$ will be determined by the particular environment in which it was grown as well as by the possibilities inherent in the germ cells which begot it. Even under the so-called standard conditions of a scientific experiment there are numerous uncontrolled variables to which any two species will react differentially. These will include such factors as time of year, sunshine, humidity, temperature, crowding, fumigation, and others. For every differentially selective factor in the environment there will tend to be a selection of $F_2$ segregates. An ideal $F_2$ is as impossible as an ideal environment.

*Pleiotropism.* One of the severe hindrances to character combination in species crosses is the pleiotropic effect of the genes by which the species differ. As GRÜNEBERG points out in his introduction to the analysis of a lethal mutation in the rat (1938), "The number of observable 'characters' in an organism is infinite. The number of genes which control development is limited. It follows that many, perhaps most, genes must not affect only one organ or character but several at a time. Their effect is manifold." GRÜNEBERG then proceeds to distinguish between "genuine pleiotropism" in which the gene exerts its manifold effect through different primary effects and "spurious pleiotropism" in which a single primary effect results ultimately in manifold effects. He concludes that the cases of pleiotropism which have been reasonably well analyzed are all spurious pleiotropism and that genuine pleiotropism if it does indeed exist will be difficult to demonstrate.

There is good evidence that "spurious pleiotropism" is one of the hindrances to recombination in the cross between *N. alata* and *N. Langsdorffii*. If a gene exerts its primary effect in more than one chain of de-

velopmental processes it may be predicted that other factors, including various environmental agents, will also affect both chains and as a result variation in the two end characters will be correlated even in the absence of gross genic differences, as in the $F_1$. The $F_1$ correlations will therefore

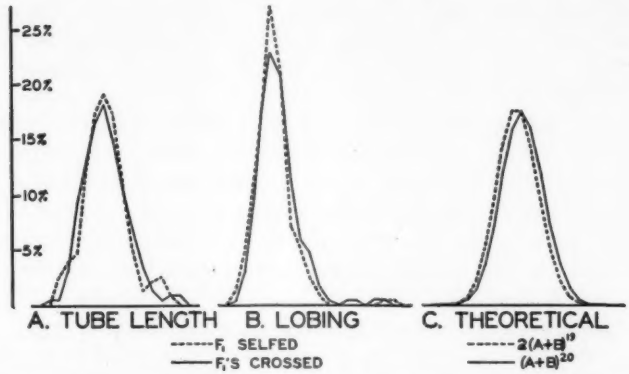


FIGURE 12.—Frequency distributions of $F_2$'s obtained by selfing an $F_1$ (broken line) or crossing two completely compatible $F_1$'s (solid line). Left to right: (A) actual results for tube length, (B) actual results for corolla lobing, and (C) theoretical expectations for one gene per chromosome.

be a good measure of the "spurious pleiotropism" of the genes governing their development. The more highly correlated they are in the $F_1$, the greater will be the proportion of common developmental processes and the less will be the chance that a gene may affect one character without affecting the other in a corresponding way.

The characters studied in this experiment which showed significant $F_1$ correlations (table 1) were style length vs. corolla tube length, $r = +.89$, and lobing vs. limb width, $r = +.31$. The high correlation of style length

TABLE 1

*Correlation coefficients in the $F_1$ and the $F_2$ of width of corolla limb (m.l.), corolla lobing (m.l./a.s.), corolla tube length (t.l.), and style length (s.l.).*

| | $F_1$ | $F_2$ |
|---|---|---|
| $\frac{\text{m. l.}}{\text{t. l.}}$ | $-.0255 \pm .1191$ | $.7196 \pm .0299$ |
| $\frac{\text{m. l./a. s.}}{\text{t. l.}}$ | $-.0950 \pm .1181$ | $.5326 \pm .0445$ |
| $\frac{\text{s. l.}}{\text{t. l.}}$ | $.8867 \pm .0255$ | $.9553 \pm .0054$ |
| $\frac{\text{m. l./a. s.}}{\text{m. l.}}$ | $.3105 \pm .1077$ | $.7186 \pm .0300$ |

and corolla-tube length in the second generation of this cross was commented upon by EAST (1916), though he apparently did not note that it was almost as high in the genicly uniform $F_1$ as in the genicly diverse $F_2$. The fact that the $F_2$ correlation is only slightly higher than that for the $F_1$ would suggest that *N. alata* has few if any germplasmic differences making for a longer style in addition to those which affect the corolla. A hybrid with the corolla as short as that of *N. Langsdorffii* and a style as long as that of *N. alata* (or *vice versa*) is therefore a physiological impossibility.

Similarly, as might be expected, the lobing of the corolla, is to a certain extent dependent upon its size. The larger the corolla, the greater will be the proportional depth of the sinuses. In this case, however, the $F_2$ correlation (+.71) is much higher than the $F_1$ (+.31). It is evident therefore that *N. alata* not only has genes which make the limb proportionately larger (and at the same time somewhat more lobed) but also other genes which tend to accentuate the lobing.

The growth of the limb and the amount of its lobing seem to be physiologically independent of the development of the tube. At least they do not have enough developmental processes in common so that fluctuations in one, under ordinary conditions are accompanied to any appreciable extent by corresponding fluctuations in the other.

Genuinely pleiotropic genes, affecting both corolla tube length and limb width or lobing, cannot be excluded as a possibility though at the present time there is no clear-cut evidence either for or against such an assumption.

"Spurious pleiotropism" certainly imposes a severe restriction upon character combinations of the plant as a whole. *Nicotiana Langsdorffii* is generally in both leaf and flower a smaller, coarser, stubbier species than *N. alata*. Such general specific differences, affecting aspect and texture throughout the organism, must rest upon factors which are "spuriously pleiotropic" and which therefore exert similar influences in different developmental sequences (ANDERSON and WHITAKER 1934). As might be expected, therefore, the hybrids as a whole present a picture of correlated intermediacies. Although difficult to measure or even to describe in exact terms, each hybrid exhibits a series of correlated characters affecting texture, aspect, leaf-form, flower shape, and inflorescence development. Furthermore in the group as a whole it can be seen that the general degree of intermediacy tends to be the same throughout any particular plant.

*Linkage.* The following discussion of the hindrance to recombination caused by linkage has been worked out on the basis of the multiple factor hypothesis. In the opinion of the author, this hypothesis, however useful it may be as a working approximation, rests upon slight and indirect

evidence. One might better say that it has never been disproved than that it has been conclusively established. Though it will be used in its simplest form in the following discussion, certain modifications would fit the facts quite as well. The better part of the evidence for the multiple factor hypothesis is merely evidence for intranuclear inheritance. From what little is known of the morphology and physiology of chromosomes it would seem possible that in addition to genes there might also be some ground substance (such as long protein chains) which were chemically uniform throughout a chromosome but which differed in species or races. So far as the author is aware there is as yet no evidence, even remotely critical, which will decide between the hypothesis of "genes alone" and that of "genes plus ground substance."

The data reported above contain two independent bits of evidence which, though they will not decide between the original multiple factor hypothesis and such modifications of it, are at least evidence for it or for some very similar hypothesis.

The first of these is the morphological difference between the $F_2$'s resulting from an $F_1$ selfed and those from $F_1 \times F_1$. As has been demonstrated on pp. 680 to 681 the results actually obtained are in harmony with theoretical predictions.

The second piece of evidence relates to the degree of recombination between various multiple factor characters. It will be seen from figure 13 that the three characters, tube length, limb width, and lobing, differed in the extent to which the parental values are recovered among the $F_2$'s. Tube length falls far short of reaching the parental extremes, limb width just barely does so, while in lobing the parental mean values are achieved. On the multiple factor hypothesis this would be explained as resulting from the number of essential gene differences for these three characters. The tube length differences on this hypothesis would rest upon the largest number of gene differences, and lobing upon the least. The larger the number of genes in a multiple factor character the greater is the chance of linkages with other characters. We would therefore predict that tube length and limb width would show the greatest amount of linkage and lobing and limb width the least, with the linkage between tube length and lobing being intermediate in value. The correlations of table 1 show that this is indeed the case. The result is somewhat obscured by the physiological correlation between lobing and limb width (shown by the $F_1$ correlation). Were it not for this fact the correlation would be even lower. The results are therefore in accord with the predictions of the multiple factor hypothesis.

Although no case of multiple factor inheritance has been even approximately worked out factorially, it is evident from what little is known

that a very large number of factors must be assumed. One student of the problem, RASMUSSON, goes so far as to say (1933) that the number of genes involved in such characters will prove to be nearer to 100–200 than to 2–20 and supports his statement as follows: "The Swedish group of geneticists, most of whom have been occupied for many years in practical

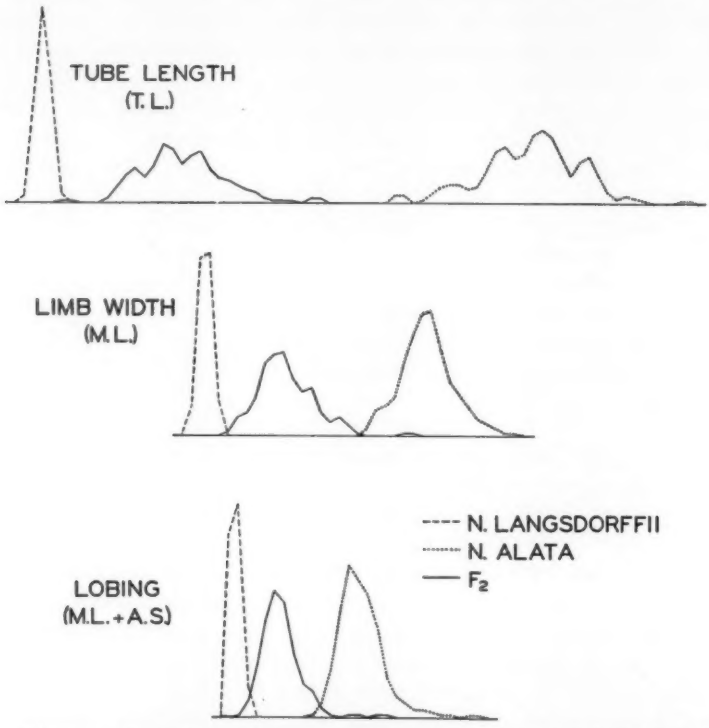


FIGURE 13.—Comparison of the degree to which the three characters, tube length, limb width, and lobing, reached the parental values in the same $F_2$. Broken line: 91 plants of *Nicotiana Langsdorffii;* dotted line: 166 plants of *N. alata;* solid line: 118 $F_2$ plants (LALA-14).

scientific breeding work, seem to be unanimous in assuming 100–200 genes for most quantitative characters in crosses between types not too closely related. In view of the great bulk of experience behind [their] opinion it seems necessary that preference be given to the assumption that a large number of genes determine the proper quantitative characters." WEXELSEN (1934), another student of quantitative characters, has an only slightly different opinion. He says: "In most cases of the plant breeder a very large number of factors are involved in characters such as yield and similar

complex traits. A fruitful genetic analysis of quantitative characters should, however, try to find forms that differ only in a few factors, and no doubt such forms can be found even for the more complex characters." "STUDENT" (1934) has calculated the number of genes necessary to explain the results of the Illinois maize selection experiments. While, as he himself is careful to point out, his calculations involve certain unverified assumptions, he concludes that they "afford some evidence" for a gene number "at least of the order of 20–40, possibly of 200–400, and not at all likely to be of the order 5–10."

TABLE 2

*Summary of published evidence on linkage between multiple factor characters and marker genes.*

| INVESTIGATOR | MATERIAL | CHARACTER | NUMBER OF MARKER GENES | NUMBER SHOWING LINKAGE | NUMBER INDEPENDENT |
|---|---|---|---|---|---|
| SAX (1923) | beans | weight | 1 | 1 | 0 |
| LINDSTROM (1926) | tomatoes | weight | 2 | 2 | 0 |
| LINDSTROM (1931) | maize | row number | 5 | 4+ | 1? |
| WEXELSEN (1934) | barley | earliness | 4 | 4 | 0 |
| RASMUSSON (1935) | peas | flowering time | 2 | 2 | 0 |
| SMITH (1937) | nicotines | corolla size | 8 | 8 | 0 |

Whether or not one makes an estimate as extreme as RASMUSSON'S it is evident that the number of factors which must ordinarily be assumed to explain breeding results is fairly high. It is a remarkable fact that linkage between marker genes and particular quantitative characters has been found in all but possibly one of the cases which have been adequately investigated in plant material (table 2). Supposedly these genes and characters have as a whole been selected more or less at random. This would indicate that genes for multiple factor characters must indeed be numerous; that on the average there must be at least one important gene for each character in every crossover segment. This would mean that the number of genes per character must be at least in the neighborhood of 50–100. If the number is indeed of this order then a remarkable secondary hypothesis can be formulated; *all quantitative characters of an organism are closely linked with one another.*

If two quantitative characters have only one gene each which are tightly linked, they should nevertheless show appreciable linkage. For instance, in a cross between *aabbccddwwxxyyzz* and *AABBCCDDWWXXYYZZ*, the first four gene pairs affecting one character and the second four another, the two characters will be linked if *a* is linked with *w*, even though the other loci are all independent. In this case the $F_1$ will be $aw/AW$, $B/b$, $C/c$, $D/d$, $x/X$, $Y/y$, $z/Z$. In the $F_2$ the *abcd*'s will be mainly limited to the

*w*'s and the *wxyz*'s to the *a*'s. If the number of genes per character is so high that it approaches one per character per crossover segment, then they are very tightly linked indeed and the linkage as a whole can only be broken after numerous generations of controlled breeding (ANDERSON 1939).

As a demonstration of the tightness of this linkage figure 14 has been prepared, diagramming the possible $F_2$ combinations (without regard to their frequencies) in a cross between two species. The diagram is restricted to a single pair of chromosomes, which differ in six essential genes affecting two different characters. The question of frequencies is not considered. The diagram illustrates all the $F_2$ genotypes which would be possible in an $F_2$ of infinite size. The $F_1$ is furthermore considered to be perfectly fertile and no structural differences affecting pairing or crossing-over have been assumed.

Factorially, the two parental chromosome types are assumed to be $a_1$, $\boldsymbol{b}_1$,$c_1$,$\boldsymbol{d}_1$,$e_1$,$\boldsymbol{f}_1$, and $a_2$,$\boldsymbol{b}_2$,$c_2$,$\boldsymbol{d}_2$,$e_2$,$\boldsymbol{f}_2$. The factors in bold face type, '***b***', '***d***', and '***f***', affect one character and '*a*', '*c*,' and '*e*,' affect the other. The species diagrammed in white, is supposed to have a minimum value for each of the two characters and the species diagrammed in black is supposed to owe its greater magnitude to the equal and additive effect of each of the six genes for which it is homozygous. (These assumptions are not necessary to the theory, but they make for a simpler and more readily understandable diagram). Each dumb-bell shaped figure in the diagram denotes a single $F_2$ genotype, black representing genes from the large species and white those from the small. As shown at the bottom of the diagram, the upper half of the "dumbbell" represents one of the chromosomes, the lower half the sister chromosome. The chromosome is diagrammatically represented in compact zig-zag arrangement $a\backslash b/c\backslash d/e\backslash f$ so that the three factors, *a*, *c*, and *e* affecting one character are pushed towards the top and the other three, (***b***, ***d***, and ***f***) are pushed towards the bottom. The smaller species is given a base value of o for each character. The larger species, by definition, will therefore carry three units of increase in each of its chromosomes, for each character, and its value on the diagram will be six for each.

The diagram is for a short chromosome which regularly has one chiasma and only one, so that only single crossovers are possible. This limits the possible kinds of gametes very severely. If the six genes were in separate chromosomes, sixty-four types of gametes would be possible. Linkage (wholly aside from its effect on frequencies), reduces the number of kinds to twelve.

The significance of the diagram in practical breeding problems is discussed below. The important point here is the demonstration of the corol-

lary of the multiple factor hypothesis outlined above; *IF THE NUMBER OF FACTORS AFFECTING A CHARACTER EQUALS OR EXCEEDS ONE PER CROSSOVER SEGMENT, THEN ALL SUCH CHARACTERS WILL BE TIGHTLY LINKED, WITHOUT REGARD TO THE FURTHER RESTRICTIONS IMPOSED BY FREQUENCIES.*

The diagram in figure 14 represents an ideal case in which there were no further restrictions imposed by gross differences in chromosome arrangement. Such cases in hybrids between species will be rather exceptional. In the actual example on which this paper is based (*N. alata*×*N. Langsdorffii*), AVERY (1938) has demonstrated that there are at least two translocations.

The chief effect of these rearrangements on character recombination will be to increase the percentage of gametic sterility and to tighten the linkage of characters coming in together from either species. AVERY has found, for instance, that in the bulk of the PMC of the $F_1$ there is an association of one quinquavalent, six bivalents and one univalent ($I_v+6_{II}+I_I$). Whether this univalent always represents the same chromosome or not (AVERY is of the opinion that it probably does) it is a chromosome in which there has been no chiasma and therefore no crossing over.

Interchanges and inversions are apparently one of the commonest barriers which separate species and races (DOBZHANSKY 1937). The effect is to suppress crossing over. In actual crosses between species and races, therefore, whether in nature or in the breeding plot, character recombination of multiple factor characters will be very severely restricted.

While linkage will hinder recombination between any two multiple factor characters, the hindrance will become proportionately greater for every additional character which is considered. The total hindrance in the $F_2$ of a species cross is therefore staggeringly great. Its minimum value is in the neighborhood of $(2n/2^n)^N$, where $n$ equals the average number of gene differences per crossover segment and N equal the number of such segments in the germplasm (Anderson 1939). In the cross used in this experiment, I am graciously informed by DR. PRISCILLA AVERY, the chiasma frequency is around eleven. If we suppose that the species differ on the average by only four genes per crossover segment (which seems a ridiculously low value) then the total hindrance is in the neighborhood of 1/2048. This means that even though we were to grow an $F_2$ so large that it occupied all the arable land on the earth, we would still be obtaining less than 1/2000 of the gene combinations possible with no linkage. In our actual $F_2$ therefore we have been obtaining only a fraction of this fraction, and in addition there have been the further restrictions imposed by pleiotropy and gametic and zygotic elimination.

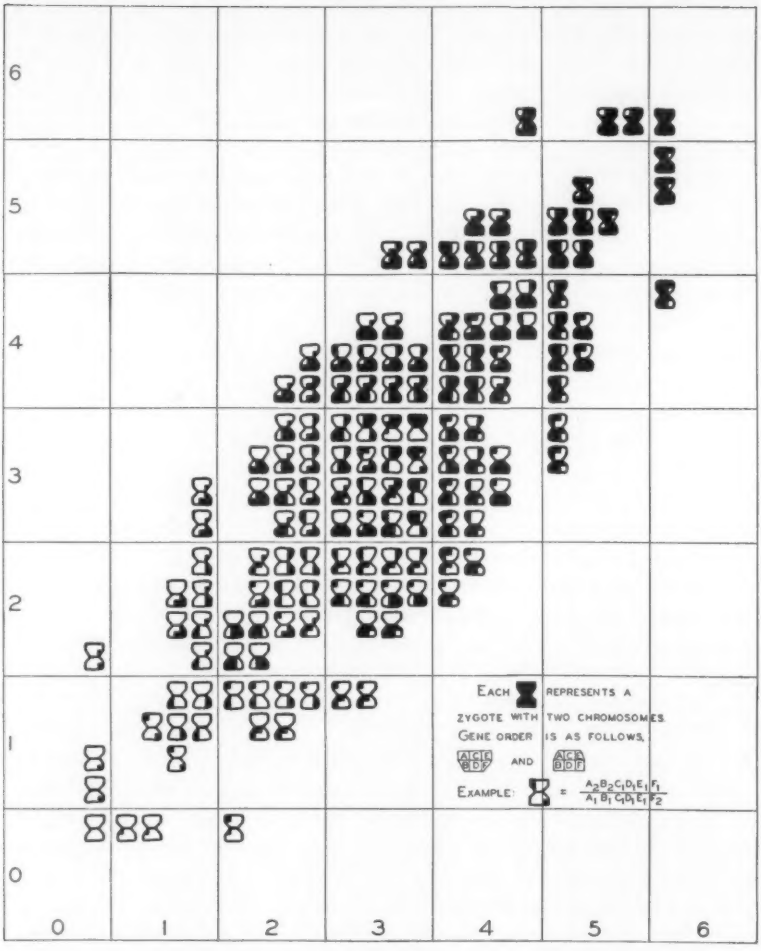


FIGURE 14.—Diagram of all the possible $F_2$ combinations (with single crossing-over) of a cross differing for six genes located in one chromosome. Genes ($a_2b_2c_2d_2e_2f_2$) derived from the "black" species are diagrammed in black, those from the "white" species ($a_1b_1c_1d_1e_1f_1$) are in outline. The genes are represented in zig-zag arrangement, those affecting the character measured on the vertical axis are above in each chromosome, those affecting the character measured on the horizontal axis are below. Each "black" gene is supposed to cause an increase of one unit and no dominance is assumed.

It was pointed out in beginning this discussion that there are at least four kinds of hindrances to character recombination in species crosses. The evidence suggests very strongly that all four are operating in this

particular cross. The evidence unfortunately is not critical enough to make even the crudest estimate of the proportion of the total restriction imposed by each of these. In particular an estimate of the relative importance of pleiotropy and linkage is of the greatest theoretical importance. The experiments reported above yield no critical evidence. The only conclusion which is justified is that the component of these two restrictive forces is very strong indeed.

Our theoretical considerations, therefore, have led to the same conclusions as did our examination of the actual data (that there are very severe hindrances to character combination in species crosses). Though the second generation of the cross between *N. alata* and *N. Langsdorffii* presents a bewildering mélange to the eye, it was found to be only a fraction, and a rather insignificant one at that, of the total imaginable combinations which might be made of the characters of the parents. It seems significant that the data and the theoretical considerations should agree and that both should yield estimates of the same order of magnitude.

It remains to ascertain if these results are general or if they are restricted to this particular cross or to similar crosses. For the flowering plants they are general. In the past five years the author has studied intensively, in the field, hybridization in the genera Tradescantia, Iris, Lindernia, Viola and Aster. In so far as character recombination is concerned these studies are in complete agreement with the results obtained in this experiment. It may therefore be concluded that character recombination in crosses between species and races is limited in the second generation to a relatively insignificant fraction of total combination. The combined effect of the various hindrances is so great that even in future generations it can be completely broken down only in terms of geological time.

## TAXONOMIC SIGNIFICANCE

Because of their bearing upon the recognition and classification of hybrids it is planned to discuss the taxonomic significance of these conclusions in a separate article. For the present the following brief outline will suggest how they may be used as a basis for morphological criteria for the recognition of hybridization in natural populations. For this purpose we may catalogue hybrids (following DARLINGTON (1937) in part) as, (A) Non-recombination and (B) Recombination hybrids. In the first of these classes will go such more or less exceptional cases as:

1. Amphidiploids, where doubling of the chromosomes eliminates interparental recombinations more or less completely.

2. Mules; hybrids in which complete or almost complete sterility prevents recombination.

3. Structural hybrids in which recombination is more or less prevented

by the peculiar architecture of the germplasm (Oenothera).

Recombination hybrids include the bulk of what are ordinarily thought of as hybrids. In such hybrids, since the time of the early hybridizers it has been known that the $F_1$ is if anything less variable than the parental species while the second generations and backcrosses show tremendous variation from individual to individual. The experiments reported above demonstrate that in spite of this variation from plant to plant, in the hybrids as a group, the characters of the parental species tend very strongly to stay together.

This fact gives us three useful morphological criteria for hybrid populations. In such populations:

1. The intermediacy of separate characters will be correlated. Hybrids intermediate in one character will tend to be intermediate in others. Hybrids which are most like either parent in any one character will tend to resemble that parent in all other characters.

2. Variation between individuals will lessen as parental character combinations are approached.

3. The novelty of the hybrids will be due to new combinations of parental characters and not to the appearance of new characters. (The only exceptions are teratological upsets in physiologically ill-adjusted hybrids.)

These criteria have already been incorporated in a technique (1936) for the study of hybrid populations. For a detailed description of their application to a particular problem see ANDERSON and TURRILL (1938) or RILEY (1938).

## PRACTICAL IMPORTANCE

The above conclusions have important bearings upon plant and animal improvement. They demonstrate that there are very severe limits to recombination in crossing species or races. Seldom or never will it be possible to incorporate one or more characters of one species with those of another species without also affecting other characters. If, for instance, we wish to incorporate the earliness of one species with the hardiness of another we shall probably have to content ourselves with combining an intermediate hardiness with an intermediate earliness. The readily possible combinations can be thought of as occupying a relatively narrow band from one parental combination, to the $F_1$, to the other parental combination. Anything away from this band can be achieved, if at all, only after a careful program of crossing and selection.

Those who have had practical breeding experience with species hybrids will recognize the truth of this generalization. Among the bearded irises, for instance, there has been a concerted attempt by iris breeders to incorporate the yellow of *Iris variegata* into *Iris pallida* without bringing along such other *variegata* characteristics as dwarf habit, foliaceous spathes, a

zig-zag stem and pencilling on the haft of the sepals. As a result of this attempt many beautiful new varieties have been produced and although the best of these might be said to approach a yellow *Iris pallida* they still show unmistakable traces of *Iris variegata* in other characters beside color.

In the genus Narcissus there has been a prolonged attempt to combine the red corona of the poet's narcissi with the flower form and yellow perianth of the trumpet daffodils. The last decade has seen the introduction of yellow hybrids with long red trumpets, but even in these recombinations the shape of the trumpet and of the perianth is still markedly intermediate.

From the standpoint of practical breeding the most important generalization to be drawn from the above experiments is that recombination in the $F_2$ is very strictly limited when the essential gene differences exceed two per crossover segment. This means that practical breeding programs need methods for deciding how the desired recombination which has started in the $F_2$ can most efficiently be continued through subsequent generations. In other words what kinds of $F_3$'s or backcrosses are most likely to lead to more desirable recombinations than have already been achieved in the $F_2$? Theoretically this selection should be comparatively simple when combining a particular character from one parent with one from the other, as for instance the size of flower of one species and the hardiness of the other. Much more difficult, theoretically, would be the selection of the most efficient $F_3$'s when the same character is coming into the cross from both sides, as for instance when attempting to select the maximum yielding strain by crossing two high yielding strains.

The diagram in figure 14 can be used to demonstrate the theoretical basis for selecting the most efficient $F_3$'s. Selfing desirable $F_2$'s may lead to extinction of desirable genes not already present, since the desirable $F_2$'s fall near that part of the recombination area where there is the greatest genotypical variation within each phenotype (see below). Crossing the best $F_2$'s back to either parent will prevent the incorporation of desirable genes not already brought in from the other parent. The most efficient crosses should be between the desirable combinations which are most like one of the parents in one of the characters with those which are most like the other parent in the other character. In the above example of an attempt to combine flower size from one species with hardiness from another the most efficient $F_3$'s should be produced by crossing the hardiest of the $F_2$'s with flowers like the large parent with the largest flowered of those $F_2$'s which were as hardy as the other parent. Such crosses should conserve desirable combinations already achieved and at the same time prevent the loss of ultimately useful genes from either species.

These same facts can be demonstrated more concretely by reference to

figure 14. If we take as the desired combination, the lower right hand corner of the correlation diagram (6, 0) then our closest possible approach to it in an $F_2$ are the combinations in cell (4, 2). Ordinary procedure would be to self these, to cross them together, or perhaps to backcross them to one of the parents. On the theory outlined above we should instead make a cross between the combination in cell (6, 4) and the one in cell (2, 0).

The theoretical results of these various procedures are illustrated in figure 15 which shows on grids having the same values as that of figure 14 all the possible combinations to be expected from (A) the cross recom-

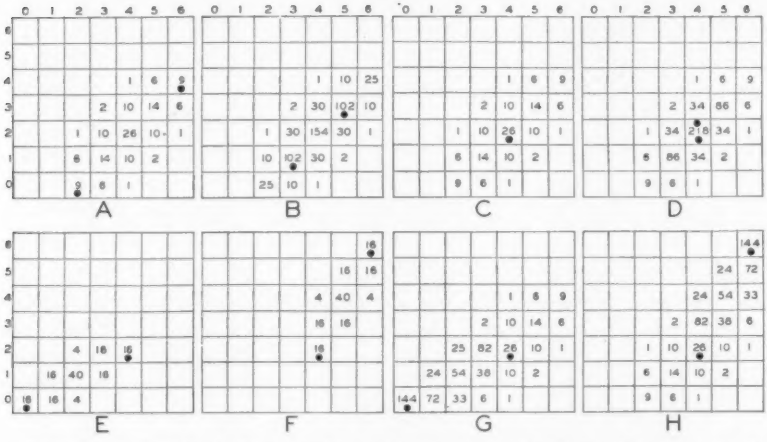


FIGURE 15.—Results of selfing, crossing, and backcrossing different $F_2$'s of figure 14. Black dots show the position of the parents in each case. Numbers indicate the number of times a combination in each cell can be obtained (irrespective of cross-over frqeuencies).

mended above, (C) selfing one of the desirable $F_2$'s, (D) crossing two of the desirable $F_2$'s, and (E, F, G, and H) crossing the desirable $F_2$'s back to either parent.

Reference to figure 14 will show that as one approaches the center of the diagram the genotypic variation within each phenotype increases enormously. Cell 3, 3 for instance has eight genotypes all of which are heterozygous. This property will go up exponentially as the number of chromosome pairs is increased, so that selections from in or near this area will be very unreliable in actual practice. Even with one chromosome pair, however, it will be seen that the recommended cross gives as high a proportion of combinations in the desired direction as do the luckiest selections from the best combinations of the $F_2$.

The problem of the most efficient $F_2$ selections becomes much more difficult when the same multiple factor character is coming in from each

side of the cross. There is then no morphological indication of the particular germplasm of each $F_2$ individual. Multiple factors may be thought of as markers for the bulk of the germplasm in the same way that single factor characters are markers for a particular piece of a particular chromosome. A cross in which an attempt is being made to combine the high yielding capacities of both parents cannot be treated in this way because the marker (yield) enters the cross from both sides.

This problem has been carefully worked out by RICHEY (1927) and by RICHEY and SPRAGUE (1931) who by a series of backcrosses and selections have solved the problem of conserving the recombinations already achieved without losing genes of potential value. The only defect in their system (which has proven of practical value) is that it is slow and costly. On the basis of the theory outlined above, the use of multiple factor characters as markers might be suggested. Theoretically at least it should make for greater efficiency. The procedure would be as follows: Two multiple factor characters should be chosen which differentiate the two parental lines. These might conceivably be any character such as leaf width, height, tassel branching, cob shape, etc. Characters easily observed, resting upon a large number of factors, and having no direct effect upon yield would probably be the most useful.

As an example suppose that the two strains are characterized as follows: strain A, very branched tassel, narrow leaves; strain B, slightly branched tassel, wide leaves. Then, if the number of factors affecting yield is indeed in the neighborhood of 100–200 as RASMUSSON (1935) estimates, the most efficient choices among the high yielding $F_2$'s would be those which come closest to combining one of the marker characters of one parent with the marker character of the other. To be specific: if among the high-yielding backcrosses to A, there could be selected a line with tassels as branched as those of A but with a wider leaf, and from those to B a line with leaves as wide as those of B but with a more branched tassel, a cross between these should give better and quicker results than a cross between parents selected on the basis of yield alone.

Theoretically, the procedure as recommended here is an advance on that recommended by RICHEY and SPRAGUE. They select for backcrosses resembling the non-recurrent parent in toto. We advise selecting backcrosses which come nearest to combining a character from the non-recurrent parent with a character from the recurrent one. Their method would select backcrosses having the most and the largest chunks of non-recurrent germplasm. Ours would select backcrosses in which the two germplasms had been broken into the smallest pieces and most completely intermingled. If the morphological selection had been made concurrently with those based on yield it is among such backcrosses that permanent improvement is to be looked for.

SUMMARY

1. The study of total recombinations is presented as a technique for analyzing the genetics of crosses between species or races.

2. The cross between *Nicotiana alata* and *N. Langsdorffii* was selected for such a study because it was (a) easy to grow, (b) highly fertile in the $F_1$, and (c) between morphologically very dissimilar parents.

3. The differences between these two species are tabulated. Reasons are advanced for limiting the study to differences in corolla shape and color.

4. The $F_2$ recombinations of tube-length and limb-width, of tube-length and degree of lobing, of limb-width and degree of lobing, and of corolla-length and style-length are described and discussed. It is shown that in each case there is not even an approach to free recombination of the parental values. Instead recombination is found to be restricted to a narrow segment of the total, running from combinations more or less like one parent to combinations more or less like the $F_1$ to combinations more or less like the other parent. This generalization is shown to hold when recombinations in shape and color are considered simultaneously.

5. A mathematical formula is developed for generalizing the results obtained from studying recombinations two at a time. From this formula it is estimated that the actual character recombinations of the above $F_2$'s are less than 1/64 of the total imaginable recombinations.

6. As a graphical demonstration of this assertion, the extreme recombinations of the $F_2$'s are compared with the extremes to be expected under total recombination (figures 9 and 10). It is seen that though the $F_2$'s present a bewildering variation from plant to plant they nevertheless are decidedly uniform by comparison with total recombination.

7. In the light of these results, the known hindrances to character combination are discussed under the following headings: (a) gametic elimination, (b) zygotic elimination, (c) pleiotropism, and (d) linkage. Evidence is brought forward to show that all of these operated to hinder recombination in the above cross.

8. The hindrances imposed by linkage of multiple factor characters are found to be severe. It is shown that if the number of factors for each such character is as large as is ordinarily postulated then all the multiple factor characters of an organism will be tightly linked with each other, without regard to the further restrictions imposed by frequencies of recombinations.

9. Theoretical considerations therefore lead to the same conclusion as did the actual data. Recombination in crosses between species and races is limited in the second generation to a relatively insignificant fraction of total recombination.

10. The application of these conclusions to practical breeding problems is discussed. The limitations of $F_2$ character recombination are shown to impose a severe handicap and methods are described for selecting the most efficient parents for further breeding from among the $F_2$'s.

11. The taxonomic significance of these conclusions is deferred to a future publication. It is shown that they may be used as the basis for morphological criteria for the recognition and measurement of hybridization in natural populations.

12. The above points summarize the main body of the paper. There are, however, a number of digressions to which reference may be made.

a. The morphological consequences of gametic elimination through interaction of self-sterility alleles (p. 681).

b. A description of the effects of pleiotropy in hybrid populations (p. 684).

c. Two new pieces of evidence favoring the multiple factor hypothesis or some very similar hypothesis.

d. The suggested use of multiple factor characters as markers for the bulk of the germplasm in the same way that genes are used to mark particular points (p. 695).

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# STRUCTURE AND VARIATION OF THE CHROMOSOMES IN DROSOPHILA ALGONQUIN

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DROSOPHILA *algonquin*, described by STURTEVANT and DOBZHANSKY (1936b), belongs to the *affinis* group of the genus Drosophila. It was originally found in a few localities in the northeastern United States, but it has recently appeared in a collection taken as far southwest as central Texas. Figure 1 shows the known distribution of the species.

It is the purpose of this paper to give an account of the structure of the chromosomes of *Drosophila algonquin* and to describe the nature and distribution of variation in gene arrangement within them.

## THE STANDARD CHROMOSOME STRUCTURE

*Drosophila algonquin* has five pairs of chromosomes. Seen at metaphase in the giant ganglion cells of the female larva these appear as a pair of large V-shaped X chromosomes, a pair of V-shaped autosomes, two pairs of J-shaped autosomes, the smaller one with an almost terminal spindle attachment, and a pair of dot-like autosomes (Plate 1).

In the salivary gland cells of the larva the nucleus contains seven large and one very small chromosome strands (Plate 1). The maps representing the disc patterns in each of these strands shown in Plate 1 are composites of a number of camera lucida drawings. After the manner of BRIDGES (1935), the chromosome arms are divided arbitrarily into numbered sections to facilitate the study of gene arrangements in chromosomal aberrations.

The correspondence between the strands seen in the salivary gland cells and the metaphase chromosomes, although not securely established, is suggested by a number of facts. Two of the arms appear definitely paler in preparations made from male larvae than in those from females. It is concluded that these represent the X chromosome, which is haploid in the male and thus may be expected to be less intensely stained in the male nuclei than in the female ones. The association of the remaining arms into chromosomes is inferred from their most frequent associations when the chromocenter has been broken up by pressure on the cover glass (BAUER 1936). The longest autosome strand quite frequently separates from all the others and retains at its base only a small bit of rather darkly staining heterochromatin. It is concluded that this strand alone with its heterochromatin attachment represents a chromosome. This is designated the A chromosome. Two of the autosomal strands frequently separate from

the others together with only a small amount of heterochromatin between them. These are considered to consitute the B chromosome. The remaining two long strands appear to make up the C chromosome. Here, however, the mass of heterochromatin between the two arms is much greater than

FIGURE 1.—Distribution map for *Drosophila algonquin*. The black circles represent places from which strains of this fly were studied as to chromosome structure and variation. The circles with X's in them represent places from which *Drosophila algonquin* has been collected but from which no strains were available for this study.

in the B chromosome, and it often has one or more of the other strands attached to it. The very small chromosome strand appears to be independent and most probably represents the dot-like chromosome seen in the metaphase plate.

The short limb of the X and the long limb of the C chromosome each frequently shows a basal section lying in the chromocenter. This appears

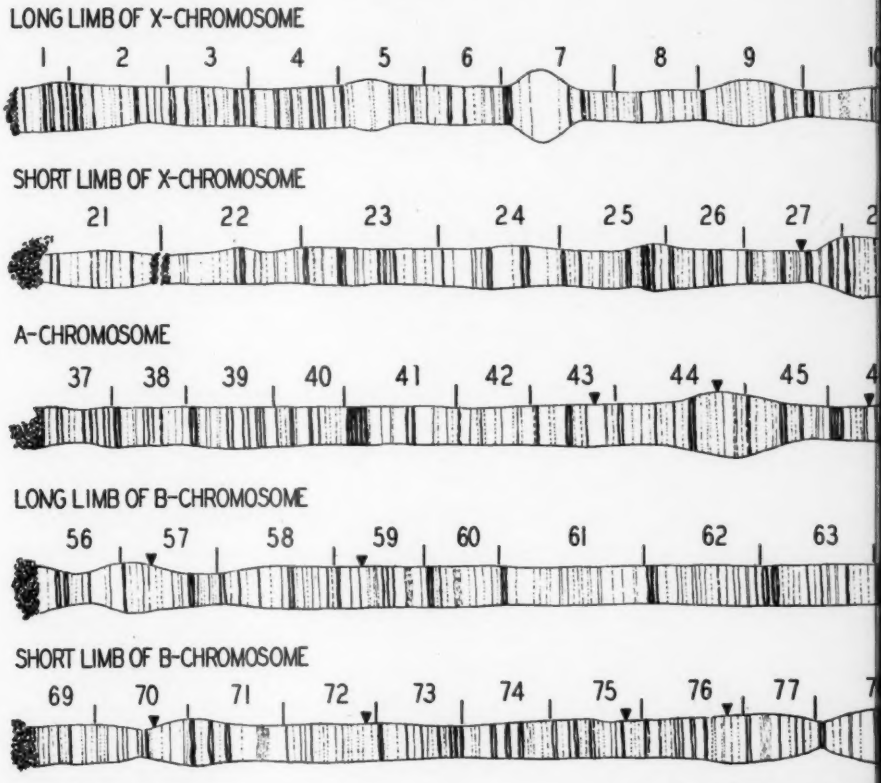
LONG LIMB OF X-CHROMOSOME
1 2 3 4 5 6 7 8 9
SHORT LIMB OF X-CHROMOSOME
21 22 23 24 25 26 27
A-CHROMOSOME
37 38 39 40 41 42 43 44 45
LONG LIMB OF B-CHROMOSOME
56 57 58 59 60 61 62 63
SHORT LIMB OF B-CHROMOSOME
69 70 71 72 73 74 75 76 77



PLATE 1.—The salivary gland chrom
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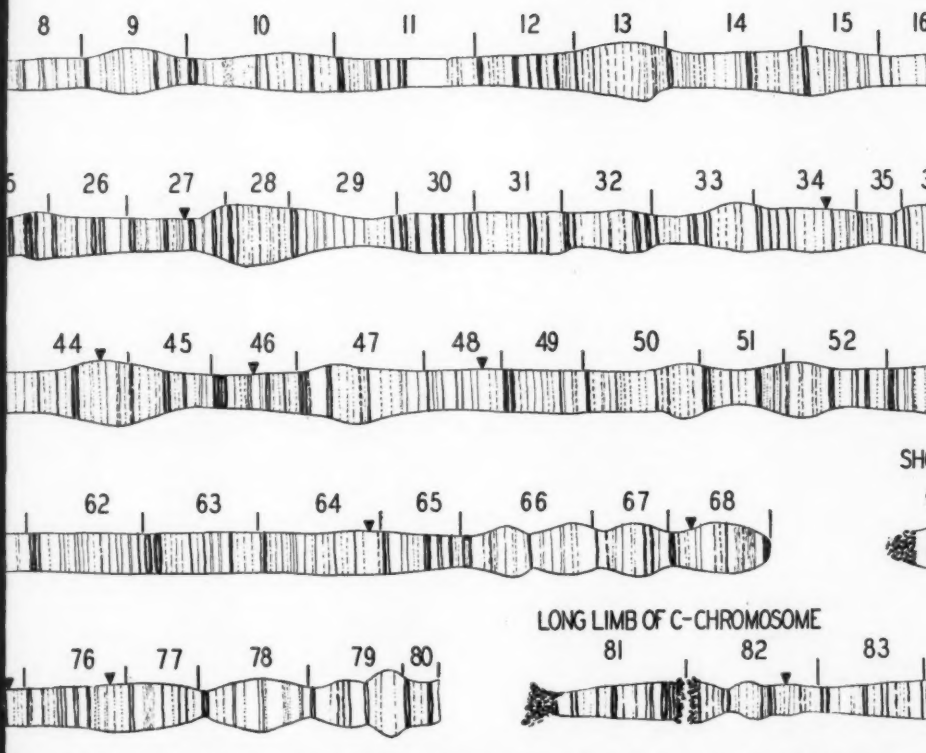
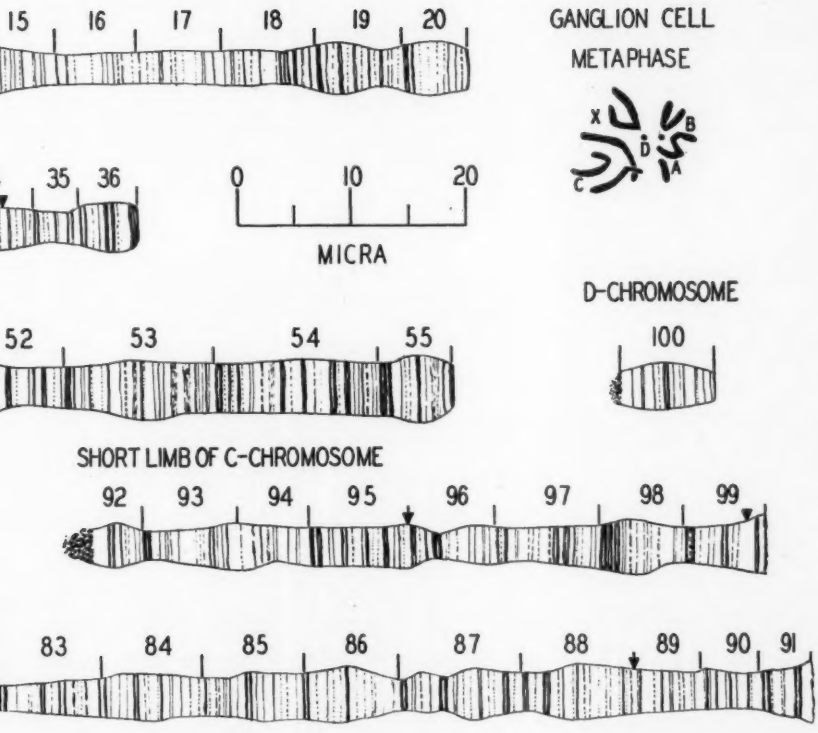



TE 1.—The salivary gland chromosomes of *Drosophila algonquin*. The sequence is the Standard used in this study. Th
indicate the breakage points of the inversions described in the text.




his study. The black triangles

to be due to a tendency for both ends of this section to be attracted to heterochromatin. Each of these is shown in the maps as a section separate from the rest of its arm with heterochromatin adhering to the point of separation.

## INVERTED SECTIONS IN WILD STRAINS

Each strain of flies studied was derived as the progeny of a single female captured from the wild. The distribution of these strains is given in the first two columns of table 1.

TABLE 1

| LOCALITY | STRAINS | SEQUENCES BESIDES STANDARD |
|---|---|---|
| Aldrich, near Austin, Texas | 39 | None |
| Belfast, Maine | 1 | X-1, A-2, B-3, C-1, C-2 |
| Chautauqua, New York | 6 | A-1 |
| Franconia Notch, Twin Mountain, Gale River, N.H. | 5 | X-1, A-1, A-2, B-3, C-1, C-2 |
| Gray, Maine | 2 | A-2, B-1, B-3, C-1 |
| Ithaca, Varna, N.Y. | 5 | A-1, B-3 |
| South Amherst, Mass. | 1 | A-1, B-3, C-1 |
| Valmorin, Quebec | 1 | B-3 |
| Woods Hole, Mass. | 3 | A-1, A-2, B-1, B-2, B-3 |
| Wooster, Ohio | 2+13 wild ♂♂ | A-1, B-3 |

A gene arrangement found in all these localities was chosen arbitrarily as the Standard. The strains were tested for variations in gene sequence by mating them to flies of the Standard type. Acetocarmine preparations of the salivary glands of the offspring larvae of these matings were made, and the nuclei were examined for structural heterozygosity. A number of sequences differing from the Standard by inversions were discovered in this way.

No inversions were found in the long limb of the X chromosome. In the short limb of the X there was found a subterminal inversion (figure 2A). The non-standard sequence involved in the configuration was called X-1. The inversion breakage points occur in sections 27 and 34 and are indicated by triangles in those sections in Plate 1. Sequence X-1 occurred in strains from Belfast and Twin Mountain (near Franconia Notch).

Two sequences besides Standard occur in the A chromosome. Compared to the Standard one of them involves a single inversion in the central region of the chromosome (figure 2B). This sequence is designated A-1 (break points in 44 and 48, Plate 1). It has been found in the following localities: Wooster, Chautauqua, Ithaca, Varna (near Ithaca), South Amherst, Woods Hole, and Twin Mountain. The other sequence, called A-2 (figure 2C), differs from the Standard by two overlapping inversions in the middle part of the chromosome. This has been found in Woods Hole,

Twin Mountain, Gray, and Belfast. A structural heterozygote carrying the sequences A-1 and A-2 exhibits a single loop configuration, indicating that these two sequences differ from each other by a single inversion (break points in 43 and 46, Plate 1).

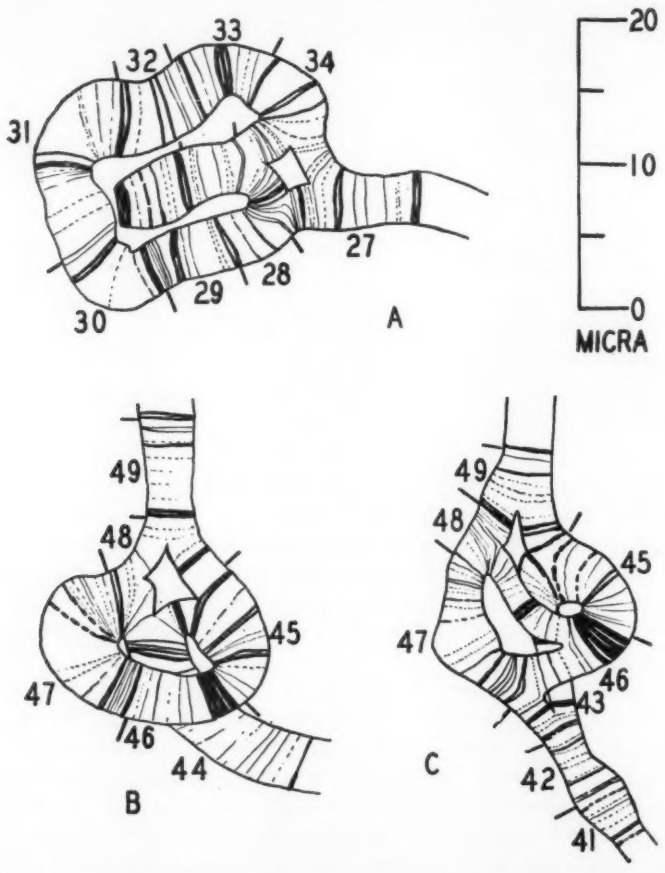



FIGURE 2.—Inversion configurations in the salivary chromosomes of individuals heterozygous for the Standard sequence and these inversions. A. Standard/X-1. B. Standard/A-1 C. Standard/A-2.

It has been demonstrated (STURTEVANT and DOBZHANSKY 1936a; DOBZHANSKY and STURTEVANT 1938) that the simplest explanation of the relationship between two gene sequences differing from each other by two overlapping inversions is that one of them arose from the other through

the formation of an intermediate sequence differing from either by a single inversion or that both arose from the intermediate sequence. The most likely interpretation of the condition encountered in the A chromosome of *Drosophila algonquin* thus becomes that the sequence A-1 is a phylogenetic connecting link between Standard and the sequence A-2. This relationship is shown diagrammatically in figure 3. Which of these three sequences is

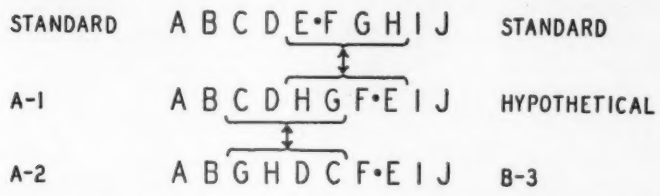



FIGURE 3.—Diagram showing the relationship of two gene sequences differing from each other by two overlapping inversions. The labels at the left pertain to the A chromosome of *Drosophila algonquin*. The spindle attachment of this chromosome may be considered to be either in the vicinity of A or of J. The labels at the right pertain to the B chromosome of *D. algonquin*. The spindle attachment of this chromosome is represented by the dot between E and F

the ancestral one is a matter of conjecture. From this study Standard appears to be much more widespread than either A-1 or A-2. Moreover, A-1 occurs in more localities and over a wider range than A-2. It is therefore possible that both Standard and A-1 are of earlier origin than A-2, and Standard earlier than A-1, allowing for the following phylogeny: Standard→A-1→A-2.

In the long limb of the B chromosome there is found a large inversion (figure 4A). This sequence is designated B-1 (break points in 59 and 68, Plate 1). It is found in Woods Hole and Gray. Another sequence in the B-chromosome differs from the Standard by an approximately median inversion in the short limb (figure 4B). This sequence, B-2 (break points in 70 and 76), has been found only at Woods Hole.

Involving both the long and the short arms of the B chromosome there are two overlapping inversions, one of which includes the spindle attachment (figure 4C). This rearrangement may be expected to be noticeable as a visible alteration of the shape of one of the ganglion cell chromosomes, since the length relationship of the two arms of B is quite drastically changed by it. The ganglia of larvae homozygous for this sequence were examined. Here it was found that there was no V-shaped autosome, but a J-shaped chromosome in its place (figure 4D). STURTEVANT and DOBZHANSKY (1935b) originally described the chromosome complement of *Drosophila algonquin* as containing no V-shaped autosome. The specimens of *D. algonquin* examined cytologically by STURTEVANT and DOBZHANSKY

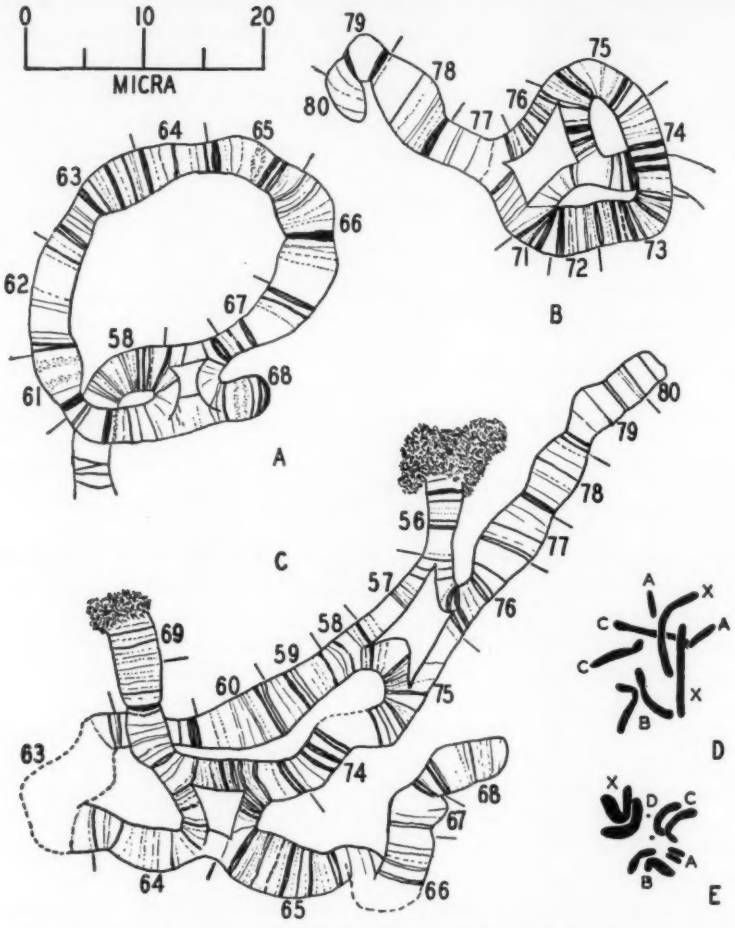



FIGURE 4—Inversion configurations. A. Standard/B-1 B. Standard/B-2 C. Standard/B-3. The chromosome may be considered to be continuous through heterochromatin between sections 69 and 56, although in this particular figure the heterochromatin connection is broken. D. Ganglion cell metaphase plate from an individual homozygous for sequence B-3. Compare the shape of the B chromosome here with that of the B chromosome in the metaphase plate shown in Plate 1. E. Ganglion cell metaphase plate from a Standard/B-3 individual, showing the two kinds of B chromosome. The scale of micra for this figure pertains only to the salivary chromosome drawings. The ganglion cell chromosomes are drawn on a slightly larger scale.

were most probably homozygous for the gene arrangement differing from my Standard through the inversion involving the spindle attachment. This sequence is called B-3. The breakage points of the spindle attachment

inversion are in 57 and 75, and the breakage points of the inversion that overlaps it are in 64 and 72. Sequence B-3 is quite widespread, occurring in the following localities: Wooster, Varna, Valmorin, Woods Hole, South Amherst, Twin Mountain, Gale River, Gray, and Belfast.

An individual heterozygous with respect to an inversion across the spindle attachment gives rise to a number of inviable offspring due to crossing over within this inversion resulting in duplications and deficiencies in the chromosome involved (STURTEVANT and BEADLE 1936). Thus, a gene sequence arising in a wild population through an inversion including the spindle attachment may be expected to be at a disadvantage in the face of natural selection, since heterozygotes for this new sequence and the prevailing sequence will produce inviable offspring. However, if the spindle attachment inversion is overlapped by another inversion, crossing over within it may be reduced to the extent that the above-mentioned disadvantage is virtually overcome. Sequence B-3 of *Drosophila algonquin* differs from the Standard by an inversion involving the spindle attachment and an overlapping inversion. Apparently B-3 and Standard are at little disadvantage in the presence of each other, since this study shows that they exist together in a number of places. Since B-3 and Standard differ from each other by two overlapping inversions, the most probable relationship of these two sequences is through an intermediate sequence differing from each of them by a single inversion. Such a sequence has not been found. The supposed relationship of Standard and B-3 is diagrammed in figure 3. The Hypothetical sequence differs from Standard by the spindle attachment inversion alone. Thus, in spite of the apparent attending disadvantage, it seems that Standard arose from Hypothetical and persisted or that Hypothetical arose from Standard and remained long enough to give rise to B-3. The supposition that this evolutionary step is difficult is supported by the fact that until now no inversion across the spindle attachment had been found in wild populations of Drosophila, although such chromosomal aberrations have been derived by X-rays (CATCHESIDE 1938).

The inverted region of sequence B-3 overlaps both the inversion of B-1 and that of B-2. Thus, Standard is a phylogenetic connecting link between B-3 on the one hand and B-1 and B-2 on the other. The small distribution areas of B-1 and B-2 may mean that these sequences are more recent than Standard.

In the long limb of the C chromosome there is a median inversion (figure 5A). This is designated C-1 (break points in 82 and on the 88–89 boundary). It is found in South Amherst, Twin Mountain, Gray and Belfast. The short limb of the C chromosome has an inversion that is almost terminal (figures 5B and 5C). This sequence, C-2 (break points at 95–96 boundary and in 99), has been found in Twin Mountain and Belfast. Only the two

most terminal discs indicated on the standard map appear not to be inverted, and pairing of these discs is usually not achieved.

An outline of the occurrence of the various gene sequences described above is given in table 1. As already stated, the Standard sequence for each chromosome occurs in all the localities from which flies were studied. It is of interest to note that this arrangement alone was found in the 39 strains from Aldrich, Texas. However, one need not conclude that the Austin region population of *Drosophila algonquin* is homogeneous for gene sequence. According to PROFESSOR J. T. PATTERSON, who made the Texas

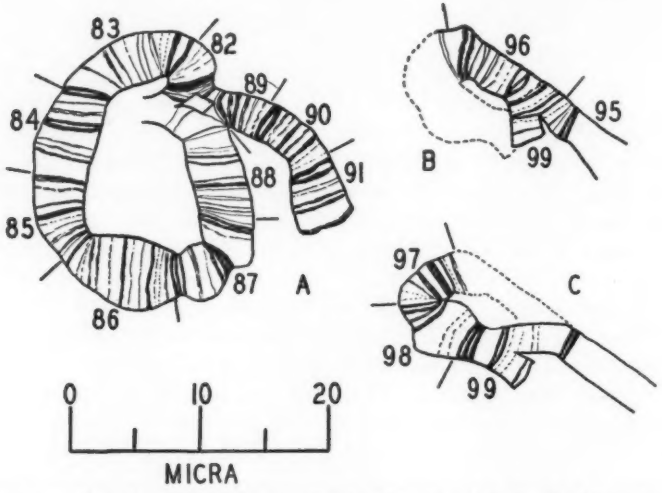



FIGURE 5.—Inversion configurations. A. Standard/C-1 B. and C. Standard/C-2, the same configuration at two different foci. This configuration is typical for this inversion heterozygote. Sometimes the last two discs of section 99 pair completely.

collection, *D. algonquin* is not common in this region and the individuals that were collected here were all gotten within a rather small area. Moreover, the region surrounding Austin is quite different climatically from the northeastern United States, where *D. algonquin* is prevalent. Thus, it appears that the Austin region is probably at the limit of the distribution of the species, and it is likely that the species all but disappears from the region at certain times. The collection used in this study may be the progeny of a few pairs of flies that persisted through periods of scarcity for the species in this region. Another possible explanation is that these flies represent the progeny of a few females introduced here artificially from the north. At any rate, a more thorough study of the *Drosophila algonquin* population of Texas and the territory intervening between Texas and the

other places where *D. algonquin* has been found needs to be made in order to establish the nature of the Texas population of the species.

In this study all the gene sequences besides Standard, A-1, and B-3 were found only in the material from Maine, New Hampshire, and Massachusetts, which also contained these sequences, although as many strains were studied from New York and Ohio as from these places. This suggests that these gene arrangements are more or less confined to the New England region and that the species is more variable as to gene sequence in this region than it is farther west.

## COMPARISON OF DROSOPHILA ALGONQUIN WITH OTHER SPECIES

A comparison of the drawings of the *D. algonquin* chromosomes with the chromosome drawings for *D. melanogaster* (BRIDGES 1935), *D. pseudoobscura* and *D. miranda* (DOBZHANSKY and TAN 1936), and *D. virilis* (HUGHES 1936) shows no striking similarities in the disc arrangement between the chromosomes of *D. algonquin* and those of the above-mentioned species. On the other hand the chromosome drawings for *D. azteca* (DOBZHANSKY and SOCOLOV 1939) and *D. athabasca* (NOVITSKI unpublished), both of which belong to the *affinis* group of Drosophila, suggest some similarities between the chromosomes of these species and those of *D. algonquin*. Whereas, *D. algonquin* has seven chromosome strands, *D. azteca* has been shown to have only six (DOBZHANSKY and SOCOLOV 1939). *D. athabasca* appears to be like *D. algonquin* in having seven strands (NOVITSKI unpublished). The following regions of the salivary chromosomes are similar in *Drosophila algonquin* and *D. athabasca:* the distal ends of the long limb of the X, of the A chromosome, of the short limb of B, and of the long and short limbs of C; the basal parts of the long and short limbs of C; and the D chromosome. The same homologies appear to hold between *D. algonquin* and *D. azteca*, except in the case of the long limb of C, which is not present at all as such in *D. azteca*.

It has recently been found that *Drosophila algonquin* may be crossed with *Drosophila athabasca*. This is the second interspecific cross known within the *affinis* group, since *D. athabasca* has already been crossed with *D. azteca*. Although the salivary chromosomes of the *athabasca-algonquin* hybrids have been studied only superficially as yet, there have been observed cases of pairing in the distal region of the long arms of C and in the distal region of the short arms of C.

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## SUMMARY

1. *Drosophila algonquin* has five pairs of chromosomes. The salivary gland nucleus of this species contains seven long and one very short chromosome arms.

2. Variation in the gene sequence has been found in all the chromosomes except the very small one.

3. An inversion in one of the autosomes includes the spindle attachment.

4. The salivary gland chromosomes of *D. algonquin* show clear resemblances to those of *D. athabasca* and *D. azteca*.

5. Hybrids between *D. algonquin* and *D. athabasca* have been obtained.

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# A GENETIC ANALYSIS OF SPECIES DIFFERENCES IN COLUMBIDAE[1]

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EVIDENCE was presented in a previous paper (IRWIN and his collaborators 1936) that certain of the antigenic characters of the red blood cells, by which *Columba guinea* is differentiated from *C. livia domestica*, had been obtained as units following, respectively, backcrosses of the species-hybrid and backcross hybrids to *livia*. Since a segregation of the cellular characters peculiar to Pearlneck (*Streptopelia chinensis*)[2] as contrasted with Ring dove (*Streptopelia risoria*) has been observed in individuals of the first and second backcrosses to Ring dove (IRWIN and COLE 1936, 1937), it would be anticipated from the results in the Columba species that isolation of the specific Pearlneck components may be accomplished. Investigations to this end are reported in this paper. References to earlier and related publications have been made previously.

## EXPERIMENTAL PROCEDURE

The cellular characters under study are recognizable at present only by immunological techniques. The details of these methods have been adequately described elsewhere (IRWIN and COLE 1936, 1937), but a brief exposition of the principles involved may be helpful. The antiserum obtained from a rabbit which has been injected with the erythrocytes of a particular species, as Pearlneck, will not by direct agglutination tests differentiate the cells of the donor species from those of any other species of the same family. That is, the highest dilution of antiserum (that is, the titer) which will cause clumping of the homologous cells will usually produce clumping also of the cells of other species of the same genus or family.

However, if the antiserum for Pearlneck, at a relatively low dilution depending upon its titer, is mixed with an excess of the cells of Ring dove or some other species, it becomes by the absorption a differentiating "reagent," or "test-fluid," which will agglutinate the homologous but not the absorbing cells. Usually, following the absorption of anti-Pearlneck serum by Ring dove cells, the titer of the absorbed serum for Pearlneck cells is reduced slightly from that of the unabsorbed antiserum.

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[2] In former papers, Pearlneck was designated as *Spilopelia chinensis*. Recently PETERS (1937) has proposed that this species be included in the genus Streptopelia.

The explanation of the above results is as follows. The injection of the blood cells into rabbits produces specific antibodies (agglutinins) for the various biochemical constituents of Pearlneck cells. Further, the interaction of Pearlneck antiserum with Ring dove cells presumably is due to the presence within these corpuscles of antigenic substances at least similar to, and probably identical with, a part of the antigenic complex of Pearlneck cells. When, therefore, Ring dove cells are mixed with Pearlneck antiserum, the agglutinins specific for those cellular substances which are *common* to the two species are adsorbed on the surfaces of the cells and are thereby removed from the serum, leaving in it only those antibodies which are specific for Pearlneck (as contrasted with Ring dove).

As has been shown in reports cited above, that part of the antigenic complex of Pearlneck which is not common to Ring dove must itself be composed of several characters. The evidence for this statement is based upon the segregation of the specific Pearlneck characters in the backcross offspring.

The recognition of the different types of cells among the backcross birds is made possible by an extension of the absorption technique, in that Pearlneck antiserum, first absorbed by Ring dove cells, is further absorbed by the cells of individual backcross hybrids. These different "reagents" would then contain the agglutinins for specific Pearlneck substances which were not removed by the cells of the respective birds. For example, let us assume that Pearlneck antiserum following absorption by Ring dove

TABLE 1

*Agglutination interactions of the species-specific Pearlneck components.*

| CELLS | TITERS FOLLOWING ABSORPTION OF | | TITERS FOR THE DIFFERENT CELLS OF ANTI-PEARLNECK SERUM, FIRST ABSORBED BY RING DOVE CELLS, THEN BY CELLS OF BACKCROSS BIRDS CONTAINING, RESPECTIVELY, ONE OF THE FOLLOWING PEARLNECK CHARACTERS | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ANTI-$F_1$ SERUM BY PEARLNECK AND RINGDOVE CELLS | ANTI-PEARLNECK SERUM BY RINGDOVE CELLS | d-1 | d-2 | d-3 | d-4 | d-5 | d-6 | d-7† | d-4 d-8 | d-9 | d-11 |
| Ringdove | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Pearlneck | 0 | 7, 8 | | | | | | | | | | |
| $F_1$ | 2, 3, 4 | 7, 8, 9 | 8 | 8 | 8 | 8 | 7, 8 | 7, 8 | 7, 8 | 8 | 8 | 6, 8 |
| d-1 | 0 | 3, 4 | 0 | 3, 4 | 3, 4 | 3, 4 | 2, 3 | 3, 4 | 4 | 2, 3, 4 | 4 | 4 |
| d-2 | 0 | 2, 3, 4 | 2, 3, 4 | 0 | 3, 4 | 2, 3 | 2, 3 | 3 | 3, 4 | 2, 3, 4 | 3 | 4 |
| d-3* | ++ | 1, 2 | 2 | 1 | 0 | 1, 2 | 1, 2 | 2 | 1, 2 | 2 | 1 | 2 |
| d-4 | 2, 3, 4 | 2, 3, 4 | 2,++ | 2 | 2 | 0 | 1, 2 | 2 | 1, 2 | 0 | 1, 2 | 2+ |
| d-5 | 0 | 5, 6 | 5, 6 | 5, 6 | 6 | 5, 6 | 0 | 5, 6 | 6 | 5, 6 | 6 | 6 |
| d-6 | 0 | 2, 3, 4 | 3 | 3, 4 | 2, 3 | 2, 3 | 2, 3 | 0 | 3 | 3, 4 | 3 | 2 |
| d-7† | 0 | 2, 3, 4 | 3,++ | 3 | 2, 3 | 3 | 2 | 3 | 0 | 1, 3 | 3 | 2, 3 |
| d-4; d-8 | 2, 3 | 3, 4 | 2, 3:3 | | 2, 2+ | 2 | 2, 3 | 2, 3 | 1, 2 | 0 | 2 | 2, 3 |
| d-9 | 0 | 2, 3 | 2 | 2 | 2, 3 | 1, 2 | 1 | 2 | 1, 2 | 1 | 0 | 2 |
| d-11 | 2, 3 | 7, 8 | 8 | 8 | 7, 8 | 8 | 8 | 7, 8 | 7, 8 | 8 | 8 | 0 |

The digits represent the highest dilution of serum at which agglutination was visible microscopically; see text for explanation. Symbol: ++ = strong agglutination at the first serum dilution.

* This substance usually required a special serum for identification; see text.

† Two substances.

cells contains agglutinins for substances A to J, respectively. If, then, the cells of a particular backcross individual removed, by further absorption, all the agglutinins except those for components A and B, such a reagent would in subsequent trials agglutinate only those cells which contained either A or B, or both.

Similarly, "second absorptions" by cells which contained only a single Pearlneck substance, as A, B or C, etc., would in each case remove only the corresponding agglutinin, and the clumping of the other cells would still be by virtue of the specific agglutinin for the respective cellular characters. (As will be explained below, although the respective Pearlneck antigenic characters, except as noted, behaved as if they were definite units and presumably each may have been due to the action of a single gene, more than one gene may be concerned in the production of any or of all. The terms "character" and "agglutinin" will be used throughout this paper, although the probable complexity and possible plurality of both should be recognized.)

## IDENTIFICATION OF THE DIFFERENT CELLULAR COMPONENTS SPECIFIC TO PEARLNECK

Following successive backcrosses to Ring dove of birds selected within families for their content of different Pearlneck characters, it has been possible to identify by the procedures outlined above different constituents of the antigenic pattern of the specific Pearlneck complex (not in Ring dove). The results of immunological tests which differentiate the Pearlneck components, each from the others, are given in table 1. These Pearlneck substances are numerically designated, respectively, d-1, d-2, d-3 . . . d-11, the letter d indicating dove.

In order that the results obtained by the use of antisera derived from different rabbits in these experiments may be comparable, the dilution for each antiserum used in absorptions by Ring dove cells has been adjusted so that the last trace of agglutination of Pearlneck cells, following absorption, was usually at the eighth dilution. Thus if the first dilution of the reagent with the cells was one part serum in 45 parts of saline, in table 1 the digit $1 = 45$, $2 = 90 \cdots 8 = 5760$; the dilutions always increasing by halves. The majority of the combinations of the cells and test-fluids as given in table 1 have been made repeatedly. Only minor fluctuations have been observed in the quantitative expression of the reactions of the cells with the different reagents, and these are given whether they occurred with reagents derived from a particular antiserum, or from different antisera.

It is not proposed that slight differences in the dilution of a particular reagent, at which cells from two individuals will agglutinate, constitute

definite antigenic differences. The only sure criterion of an antigenic difference in the cells from two individuals, is that of agglutination of one kind of cell, as compared to no clumping of the other, with a particular reagent.

The second column of data in table 1 gives what may be termed the quantitative expression of the different Pearlneck substances; that is, the highest dilutions of Pearlneck antiserum, absorbed only by Ring dove cells, at which the last trace of agglutination of these respective characters was observed microscopically. The antiserum from only one rabbit, out of more than 30 immunized with Pearlneck cells, has contained an appreciable amount of agglutinin for substance d-3. It has therefore been necessary at times to use other means of detecting this substance, as will be described elsewhere.

Substances d-5 and d-11 gave the strongest reactions of these different substances. Indeed, by interaction with this reagent the cells of d-11 were indistinguishable from those of the $F_1$, since both were usually agglutinated at the same end-dilution. Because of the finding that substance d-11 produces agglutination quantitatively equal to that of the $F_1$ cells, it seems advisable to seek a different interpretation of the distribution of the antigens in the first and second backcross generations. It would be expected that d-11 would appear in approximately half the backcross offspring of any individual whose cells contained it, and on this explanation, the ratios of birds in the different backcross generations with this component present agree fairly well with those expected. Previously (IRWIN and COLE 1936), it was proposed that, in the first backcross generation, those cells giving the same quantitative expression as the cells of the $F_1$ did so by virtue of the tendency of several Pearlneck antigens to stay together, inferring a like tendency on the part of several chromosomes bearing the causative genes. Since the d-11 antigen alone would give the same effect, the previous interpretation need no longer be invoked.

The other substances generally showed the last trace of clumping (microscopically observed) between the second and the fourth dilutions of this test-fluid, and a distinction between them was impossible by this particular reagent, despite differences in their rate and type of agglutination, optimum temperature required, etc. The non-additive effect in agglutination of two or more of these substances has also been noted previously in another species cross (IRWIN, COLE and GORDON 1936).

However, when second absorptions of Pearlneck antiserum, first absorbed by Ring dove cells, were made by cells representing each of these substances, respectively, not only was there no change for each reagent in the dilution at which $F_1$ cells were agglutinated, but, likewise, very little if any change in the dilutions at which the cells bearing the individual

substances, other than those absorbing, were clumped. Thus, d-1 cells removed all of the agglutinins for that component, and for that component only, as attested by the plus reactions of the d-1 reagent with all other cellular characters. A similar statement can be made concerning each of the other reagents, except that the substance d-8 has never been obtained except in combination with d-4, and it would be expected that absorption by cells containing the combination of d-4·d-8 would remove the specific antibodies for both characters, as was observed.

Differences between components d-4 and d-9 were not always sharply defined, and not always present. These are, however, tentatively classed as two distinct substances.

The interactions of the different "reagents" and cells as given in table 1 may be briefly explained as follows. Cells representing each of the particular Pearlneck components, d-1, d-2, . . . d-11, react with Pearlneck antiserum, absorbed only with Ring dove cells, by virtue of antibodies specific for each particular component. The removal of any one of these different antibodies, by a further absorption by specific cells, still allows each of the remaining antibodies to interact with its specific substance. Whether or not each of these different substances may be single or complex can be determined only if there should be further separation of the respective characters in the offspring of backcrosses to Ring dove.

## GENETICAL FINDINGS

The cells of the species hybrid contain nearly all of the specific components of both of the parental species (IRWIN and COLE 1936), although in the $F_1$ these different substances must, if genetically determined, be produced by genes which are simplex. The ratios expected for such characters in backcrosses to either parent would then presumably simulate those expected in the usual backcross of a monohybrid, dihybrid, etc., to a single recessive, double recessive, etc., respectively. (An exception would occur if there were a tendency for two or more chromosomes bearing genes for the specific components of one species not to separate independently at reduction division.) Thus, in backcrosses to Ring dove, if the specific part of Pearlneck cells were a single component, only two types of cells would be observed in the offspring; that is, those with and those without the Pearlneck character. If only two substances, as $\overline{A}$ and $\overline{B}$, comprised the specific part of Pearlneck, four kinds of cells would be found in the backcross offspring; that is, $\overline{A}\,\overline{B}$, $\overline{A}$, $\overline{B}$, and $\overline{O}$ (the $\overline{O}$ type of cell would naturally denote the absence of both $\overline{A}$ and $\overline{B}$, and would therefore be the same as the Ring dove cells). On this basis, the number of different types of cells possible from a backcross of the $F_1$ to Ring dove would be $2^n$, in which n represents the number of Pearlneck characters.

TABLE 2

*Distribution of the specific Pearlneck characters (of table 1) in progeny of mating to Ring dove of backcross birds carrying the different substances.*

| PEARLNECK SUBSTANCE | NUMBER OF OFFSPRING WITH | |
|---|---|---|
| | SUBSTANCE PRESENT | SUBSTANCE ABSENT |
| d-1 | 22 | 29 |
| d-2 | 5 | 9 |
| d-3 | 12 | 10 |
| d-4 | 18 | 17 |
| d-5 | 17 | 19 |
| d-6 | 27 | 29 |
| d-7* | 14 | 2 |
| d-4, d-8* | 4 | 4 |
| d-9* | 3 | 2 |
| d-11 | 25 | 29 |

* Only one backcross hybrid in each mating to Ring dove.

Obviously, if each of the different antigens represented in table 1 were but one substance, the progeny of matings to Ring dove of birds carrying any one of these components would be divided into (a) those which possessed the character and (b) those which did not, simulating the results expected within a species in the backcross of a monohybrid to the recessive. A summary of tests of the different progenies is given in table 2. It will be noted that there is an approximate equality in the proportions of individuals showing one or the other of the two kinds of cells produced from each of the different matings,[3] except for that of d-7. Some of the cellular characters were represented in the matings by several backcross hybrids, others by only a single bird. In this latter category were matings involving components d-7, d-4·d-8, and d-9.

The distribution of the progeny of the bird possessing substance d-7 more nearly approximates that expected if two, rather than a single Pearlneck component were present. Such an explanation agrees with a previous proposal (IRWIN and COLE 1936, table 4) that the cells of the individual concerned ($458A_2$) carried at least two Pearlneck characters. The results (unpublished) of reciprocal absorptions and subsequent agglutinations of the cells of the progeny also point to the presence of at least two specific Pearlneck components in the cells of this backcross individual.

[3] Matings in which d-5 was present in combination with another character produced a statistically significant excess of progeny carrying d-5, usually in combination and sometimes alone. A few other combinations of two characters have not departed from the expected equality of of the four possible kinds of cells in their backcross progeny, but in these latter the numbers have been comparatively small.

Similarly, a segregation would probably have been observed in the cells of the offspring of the individual containing d-4·d-8, given adequate numbers. The eight offspring of this mating were produced over a period of two years, and d-8 was not obtained alone. Also, although a sufficient number of offspring have not been obtained as yet in the mating of d-9 to Ring dove to state definitely that this component is not divisible, a reasonable assurance of its unit-nature has been provided by the ratios of the kinds of cells produced in progeny of matings to Ring doves of birds possessing the combinations d-6·d-9 and d-9·d-10. In each of these, only four types of cells have been observed in the offspring, indicating that d-9 is a single substance.

Adequate tests were performed on nearly all of the birds of the different progenies listed in table 2, to determine whether or not the cells giving the positive agglutinations were identical with those of the respective parents. For example, the cells of all of the offspring of different birds containing substance d-1 were used in individual "second absorptions," each reagent thus produced then being tested for the complete absorption of the agglutinin for d-1 by mixing with "tester" cells for the d-1 substance. It would be expected on a genetic basis that the cells of a backcross hybrid parent would by absorption remove all antibodies for the specific Pearlneck character or characters in the cells of its backcross offspring; that is, the offspring could, barring mutation, possess no such genetic character not present in the backcross parent. However, if the cells of a particular backcross parent contained two or more antigenic substances, by virtue of one or more genes on each of two or more different chromosomes, or of two or more genes on the same chromosome each producing different effects, a separation of the antigens in either case would be possible. The latter alternative is the more probable, and a very few interactions have been observed which suggest that certain of the specific Pearlneck components listed above are not single substances. Further studies are required, however, before interpreting these exceptions as indicative of the action of more than one gene in the production of any of the several Pearlneck substances.

It is not proposed that these ten or eleven (counting d-7 as at least two substances) cellular components of Pearlneck constitute the total number which differentiate these cells from those of Ring dove. The characters described above represent major differences between the two species, in that they are all expressed at a relatively high dilution of the antiserum. What appears to be another major Pearlneck character has been isolated, and it is probable that there are many others which are as yet undiscovered.

These data show that there are at least ten biochemical characters in

the erythrocytes of Pearlneck which distinguish it from Ring dove. The segregation of these different components in the respective backcross progenies simulates that expected if each were the product of the action of a single gene. However, identical results would be obtained if two or more genes, on as many chromosomes of Pearlneck as there are specific characters, together produced each of the different substances, and if there were no crossing over in the backcross hybrids of such Pearlneck chromosomes with their (partial, at least) homologues in Ring dove.

The possibility that any two, or more, Pearlneck characters may have been separated in the backcross generations as the result of crossovers cannot definitely be excluded. However, the manner in which a separation has occurred in the progeny of backcrosses of individuals containing a combination of two such characters (unpublished data) makes it more reasonable to assume that each of these different characters is produced by one or more genes on independent chromosomes.

## THE "HYBRID SUBSTANCE"

In view of the finding (IRWIN 1932) that the cells of the hybrid between Pearlneck and Ring dove contain one or more components not found in the cells of either parent, it is of interest to determine which, if any, of the specific unit-characters of Pearlneck are associated with the "hybrid substance." Agglutination of the cells containing any of the specific Pearlneck characters, in anti-hybrid serum absorbed by both Pearlneck and Ring dove cells, would indicate that the particular Pearlneck substance was correlated with the "hybrid substance." The results of such tests are given in the first column of data in table 1.

From the reactions it will be seen that only the cells containing d-3, d-4 and d-11 contain the "hybrid substance."[4] (Substance d-3 was weakly agglutinated, if at all, by the various anti-$F_1$ sera.) Up to the present this correlation has been positive and perfect; that is, cells not containing any one of these three Pearlneck components have not been found to have the "hybrid substance," and the presence of any one of the three, alone or in combination, has assured the presence of the "hybrid" component or components. Evidence that this "hybrid substance" is divisible into two, and probably more, parts will be presented in another paper.

## SEPARATION OF SPECIFIC RING DOVE CHARACTERS IN THE PROGENY OF BACKCROSSES TO PEARLNECK

On the basis of previous work, it would be anticipated that, following

[4] Preliminary trials of a newly produced anti-hybrid serum, absorbed by the cells of both Pearlneck and Ring dove, indicate that cells bearing Pearlneck substances other than these three (d-3, d-4 and d-11) may carry also a part of the "hybrid substance." If confirmed in future work, these findings will be considered in publications to follow.

a backcross of the species-hybrid to Pearlneck, there would be a segregation in the progeny of the specific Ring dove components. Such offspring are obtained only with difficulty under our laboratory conditions. The eggs from hybrid females rarely, if ever, produce a living squab, leaving the mating of hybrid males to Pearlneck females as probably the only source of viable progeny. The Pearlneck females are extremely wild, and only an occasional individual has produced eggs. The few offspring available for testing have been hatched over a period of several years. It being very unlikely that many others will be obtained, these few observations will be presented, in order to establish definitely that the experimental findings agree with the expectations; that is, that there would be segregation of specific Ring dove components.

The individuals of family 795 in table 3 are from the mating of a species-hybrid male to a Pearlneck female, as is the single individual $778E_3$. A bird of family 795 (795V), also mated back to Pearlneck, produced family 993.

The differences between individuals in their content of specific Ring dove substances were analyzed by methods comparable to those described in this and in previous reports for determining the segregation of specific Pearlneck characters, specific *guinea* characters, etc. A summary of the tests performed is given in table 3.

The cells of each of the individuals of family 795 were agglutinated in anti-Ring dove serum absorbed only by Pearlneck cells (see second column of data, table 3). Presumably these agglutinations were produced by virtue of specific Ring dove substances, not in Pearlneck. Further absorptions of this absorbed serum, by the cells of the individual backcross hybrids listed, provided specific reagents, which, by interactions with the different cells, allow the probable number of specific Ring dove components to be estimated. Thus, the absorption by the cells of 795W removed the antibodies for itself only, whereas the cells of 795Z exhausted the agglutinins not only for its own cells but for those of 795W as well. Further, the cells of 795X removed by absorption the antibodies for itself, as well as for both 795Z and 795W.

The results as summarized in table 3 may be hypothetically explained as follows, on a minimum basis as to the number of specific Ring dove characters involved. By the above reactions, one substance ($\overline{A}$) is required in the cells of 795W, two ($\overline{A}\,\overline{B}$) in those of 795Z, and three ($\overline{A}\,\overline{B}\,\overline{C}$) in the corpuscles of 795X. Since the "reagent" for 795V agglutinates the cells of all other birds within this family, another component ($\overline{D}$) must be added to the list. Further, the antigen of 795U removes antibodies for itself only, but this substance is found also in the cells of 795H, as are the components of 795G. These latter cells, however, share the component ($\overline{A}$) of 795W;

TABLE 3

*Interactions of the cells of individuals of the first and second backcrosses to Pearlnecks with various absorbed sera.*

| TITERS AND REACTIONS FOLLOWING ABSORPTION OF | | | AGGLUTINATION OF THE VARIOUS CELLS LISTED, WITH ANTI-RING DOVE SERUM FIRST ABSORBED BY PEARLNECK CELLS, THEN BY CELLS OF EACH OF THE FOLLOWING BIRDS: | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CELLS | ANTI-$F_1$ SERUM BY* PEARLNECK AND RING DOVE CELLS | ANTI-RING DOVE SERUM BY PEARLNECK CELLS | 778$E_2$ | 795D | 795E | 795G | 795H | 795U | 795V | 795W | 795X | 795Z | 993S | 993X | 993Y | 993$K_2$ | 993$N_2$ | 993$P_2$ | 993$R_2$ | 993$C_2$ |
| Ring dove | o | 7, 8 | 6 | 4 | 3 | 6 | 5, 6 | 5, 6 | 4, 5, 6 | 6 | 4 | 4 | 5 | 5 | 5+ | 5 | 5 | 5 | 5 | 5 |
| Pearlneck | o | o | o | o | o | o | o | o | o | o | o | o | o | o | o | o | o | o | o | o |
| $F_1$ | C | 6, 7 | | ± | | 4 | 4 | 4 | + | 4 | | | | | | | | | | |
| 778$E_2$ | + | 3 | o | o | | | o | o | o | + | o | | | | | | | | | |
| 795D | ++ | 5, 6 | ++ | o | + | ++ | ++ | ++ | ++ | ++ | + | + | | | | | | | | |
| 795E | C | 6, 7 | ++ | + | o | ++ | ++ | ++ | ++ | C | ± | ± | ++ | | ++ | ++ | ++ | ++ | + | |
| 795G | O | 4, 5 | ++ | + | + | o | o | + | ++ | ++ | + | + | ++ | | ++ | ++ | ++ | ++ | + | |
| 795H | + | 5, 6 | ++ | ++ | + | + | o | + | ++ | ++ | + | + | | | | | | | | |
| 795U | + | 5 | ++ | + | + | + | o | o | ++ | ++ | + | ± | | | | | | | | |
| 795V | C | 6, 7 | ++ | o | o | ++ | ++ | ++ | o | ++ | ±, + | ± | +, ± | o | ++ | o | o | ± | ± | ± |
| 795W | o | 4 | + | o | o | o | o | ± | ++ | o | o | o | | | | | | | | |
| 795X | | 7 | | + | o | ++ | ++ | ++ | ++ | ++ | o | + | | | | | | | | |
| 795Z | | 5, 6 | | o | o | ++ | ++ | ++ | + | ++ | o | o | | | | | | | | |
| 993P | o | o | | | | | | | | | | | | | o | | | | | |
| 993S | C | 6 | | | o | ++ | | | o | | | | o | o | ++ | o | o | o | o | o |
| 993X | | 6 | | | o | ++ | | | o | | | | ± | o | ++ | o | o | o, o+ | o | o |
| 993Y | o | 1, 2 | | | o | o | | | o | | | | o | o | o | o | o | o | o | ± |
| 993$B_2$ | o | o | | | | | | | | | | | | | o | | | | | |
| 993$F_2$ | o | o | | | | | | | | | | | | | o | | | | | |
| 993$K_2$ | C | 6 | | | o | ++ | | | o | | | | o | o | ++ | o | o | o | o | o |
| 993$N_2$ | | 6 | | | o | ++ | | | o | | | | o | o | ++ | o | o | o | o | o |
| 993$P_2$ | C | 6 | | | o | ++ | | | o | | | | o | o | ++ | o | o | o | o | o |
| 993$R_2$ | | 6 | | | o | ++ | | | o | | | | o | o | ++ | o | o | o | o | o |
| 993$V_2$ | o | o | | | | | | | | | | | | | o | | | | | |
| 993$C_2$ | C | 6 | | | | | | | o | | | | | o | ++ | o | o | o | o | o |

* First dilution of the serum-cell mixture = 1:45 or 1:90.

The digits indicate the serum-dilution at which the last trace of agglutination was observed. The first serum-cell-dilution was 1:90, 1 = 90, 2 = 180, 3 = 360, · · · 8 = 11, 520; o = no trace of clumping at the first dilution.

Symbols: C = complete agglutination; ++ = marked agglutination; + = agglutination; ± = trace; o = no agglutination—all at first dilution of the serum-cell mixture.

their respective phenotypes would be, for 795U, $\overline{E}$, for 795G, $\overline{A}\ \overline{F}$, and for 795H, $\overline{A}\ \overline{E}\ \overline{F}$. Also, by the reciprocal interactions of their cells and particular "reagents," birds 795D and E are surely not identical in their cellular patterns.

The interactions of the respective reagents with the other cells show that the antigens of 795V, W and Z are present also in 795D, while those of 795V, W, X and Z are found in 795E. The presence of a different antigen must, therefore, be assumed in the cells of each of these two birds (795D and E), giving the phenotypes $\overline{A}\ B\ \overline{D}\ \overline{G}$ for the cells of 795D, and $\overline{A}\ B\ \overline{C}\ \overline{D}\ \overline{H}$ for those of 795E. Finally, if the one or more components of $778E_3$ be considered, another antigen ($\overline{I}$) would be required. This would be present also in the cells of 795D, E, H, U, V and X, requiring then a minimum of nine different specific Ring dove characters in the cells of the species hybrid to explain the results of the table.

Illustrating the principle set forth earlier in this report, the Ring dove antiserum, after absorption by Pearlneck cells, contains specific antibodies for each of the different antigens peculiar to Ring dove. As a specific example, the cells of 795D by absorption remove agglutinins a b d g i, and this reagent then gives agglutination with the cells of 795E ($\overline{A}\ B\ \overline{C}\ \overline{D}\ \overline{H}\ \overline{I}$) by virtue of substances $\overline{C}$ and $\overline{H}$ in these latter cells; with those of 795G, H, U and X by virtue of the substances $\overline{F}$, $\overline{E}\ \overline{F}$, $\overline{E}$ and $\overline{C}$, respectively. A similar analysis of the interactions of the reagent for each individual with the other cells would explain agglutination, or lack of it, for each combination of reagents and cells. Presumably, these cellular characters particular to Ring dove are produced by genes contributed by the Ring dove parent. These genes then are simplex in the species and backcross hybrids, and the phenotypes and genotypes are the same in respect to the specific Ring dove characters of the cells.

Additional evidence regarding the specificity of the "hybrid substance" is furnished by the presence or absence of agglutination of the cells of certain of the backcross birds when tested by anti-$F_1$ serum from which the antibodies for the cells of both parents had been removed (see first column of data, table 3). Of the cells of the birds of the 795 family available for testing with this reagent, five (795D, E, H, U and V) agglutinated, while those of 795G and 795W did not. Within the offspring of 795V, family 993, and exclusive of 993Y, only those individuals showing the presence of specific Ring dove components also possessed the "hybrid substance."

The findings are in accord with the specificity proposed in the production of this hybrid substance or substances. For example, the cells of $778E_3$ contained at least a part of this character, and thus all other cells in which the one or more Ring dove antigens of $778E_3$ were found should also possess the hybrid substance, as was indeed noted. Furthermore, no other cells

containing only $\overline{A}$ or $\overline{F}$, alone or together, can carry the "hybrid component." A partial test of this condition is found in that the cells of 795W, containing $\overline{A}$ alone, have no trace of this substance. It presumably is produced by the interaction of particular genes which, in each of the two species, are linked to others producing the respective specific characters, or themselves produce such effects. Further consideration of this interaction will be presented elsewhere.

## DISCUSSION

In this paper are summarized the results of experiments to obtain, as separate entities, the different cellular characters which distinguish the Pearlneck from the Ring dove species. Also given are findings which indicate that the specific Ring dove characters, not in Pearlneck, are divisible and hereditary. There seems little doubt, in view of the segregation of both Pearlneck and Ring dove specific characters as noted, that the different antigenic constituents peculiar to the cells of each species are gene-determined.

That the cellular components *common* to both Pearlneck and Ring dove are divisible and multiple is made probable, since evidence has already been presented (IRWIN 1938) that two of the unit-characters of *C. guinea*, not in *C. livia domestica*, are shared by both Pearlneck and Ring dove. A comparison of the interrelationships of the cellular components of Pearlneck, Ring dove, *C. guinea* and *C. livia*, as previously given (IRWIN 1938), would further substantiate the above statement.

If, within the Pearlneck species, each of these genes for specific Pearlneck characters has an allele with a different effect and assuming ten such genes, the number of combinations of these species-specific characters may be readily calculated. Assuming no dominance, and with each gene giving an independent expression if heterozygous, the number of possible combinations would be $3^{10}$, or 59,049. This number would be changed if there were dominance in one or more pairs of characters, or if there were multiple alleles active at one or more loci. The genes affecting the cellular pattern of Pearlneck shared with Ring dove may be equally numerous, and thus the number of possible combinations of characters (that is, the phenotypes) for the species would indeed be very great.

The suggestion has already been made (IRWIN and COLE 1936) that the antigens of the erythrocytes may be considered as more or less direct products of the gene. The finding by LANDSTEINER and LEVINE (1926) that human sperm contain the same antigens, $\overline{A}$ and $\overline{B}$, as the blood cells of the individuals tested, may be considered as very pertinent evidence to this point. That the genic effect on the cellular antigen may not always be direct is indicated by the finding of the "hybrid substance" in certain spe-

cies hybrids, not in all, and that it or substances closely related to it are found normally in the cells of certain other species (Irwin 1935).

More recently HALDANE (1938) has discussed the possibilities of the relationship of genes to cellular antigens, proposing that "The gene is a catalyst making a particular antigen, or the antigen is simply the gene or part of it let loose from its connexion with the chromosome." Further, HALDANE (1938) and the writer appear to be in agreement in advocating that advances in our knowledge of the agglutinogens should also increase our information of the nature of the gene itself.

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SUMMARY

These data show that the species-specific components of Pearlneck may be separated into so-called unit-substances as a result of backcrosses to Ring dove. At least ten specific Pearlneck characters have been isolated by this procedure, each of which is immunologically distinct from the others. Data relative to the separation of specific Ring dove characters are also included.

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# INHERITANCE OF INTERNAL ORGAN DIFFERENCES IN GUINEA PIGS

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THE present study is a comparison with respect to weight, linear measurement, and general shape of the liver, lungs, heartt, hyroids, kidneys, adrenals, and spleen of two highly inbred strains of guinea pigs.

The two strains, family 2 and family 13, are two of several lines of brother-sister matings which were started in the United States Bureau of Animal Industry in 1906. In 1925 branches of these two strains, together with several others, were brought by PROFESSOR SEWALL WRIGHT to the University of Chicago where the same system of mating has been continued. (For a detailed pedigree of family 2 see COLIN 1931, and of family 13 see STRANDSKOV 1932.)

The original stock from which the two strains were started was a genetically heterogeneous one. Therefore, merely on the basis of chance, the two strains should be different in many gene loci. At the time that the present study was started the one strain, family 2, had 24 generations of brother-sister matings in back of it and the other, family 13, had 31 generations of such matings in back of it. According to WRIGHT (1931) heterozygosity should decrease 19.1 percent per generation in a system of brother-sister matings. Hence, no matter how heterozygous the two strains were originally, each should have been more than 99 percent homozygous when the present studies were begun.

The organs which are compared were weighed and measured immediately after the animals were killed. For the thyroid, the kidney, and the adrenal the weights and the measurements are of the left organ only. At first the same procedure was considered for the lungs, but it was found that more uniform results could be obtained if the two lungs were weighed jointly.

In addition to the weights and linear measurements of the internal organs already mentioned the body weight and the body length of each animal were taken. The latter was obtained by stretching the animal out on its back immediately after it was killed and by measuring the distance from the tip of its nose to the base of its tail.

All the animals were more than a year old when they were killed. Hence, they may be considered adults. In so far as it was possible to do so the environment was kept the same for all the animals while they were developing. All of them were kept unmated in pens of six.

## RESULTS

In table 1 are given the mean, the standard deviation and the coefficient of variability of each measurement taken. In table 2 are given the proba-

TABLE 1

*The mean, the standard deviation and the coefficient of variability of the internal organs of two highly inbred strains of guinea pigs.*

| | FAMILY 13♂♀ | | | FAMILY 2♂♀ | | | FAMILY 13♂ | | |
|---|---|---|---|---|---|---|---|---|---|
| | $\bar{V}$ | $\sigma$ | CV | $\bar{V}$ | $\sigma$ | CV | $\bar{V}$ | $\sigma$ | CV |
| Weight Body | 992.2* | 99.32 | 10.01 | 793.1 | 68.93 | 8.69 | 1044.5 | 68.61 | 6.57 |
| Length Body | 31.36 | .65 | 2.07 | 29.74 | .63 | 2.12 | 31.76 | .49 | 1.55 |
| Weight Liver | 34.59 | 6.59 | 19.05 | 27.29 | 4.39 | 16.08 | 33.70 | 3.89 | 11.54 |
| Weight Lung | 7.33 | .90 | 12.25 | 5.19 | 1.09 | 20.97 | 7.23 | .94 | 12.96 |
| Weight Heart | 2.34 | .29 | 12.40 | 2.09 | .27 | 12.90 | 2.42 | .33 | 13.47 |
| Weight Thyroid | .076 | .022 | 28.62 | .060 | .010 | 16.97 | .078 | .017 | 21.92 |
| Length Thyroid | .97 | .10 | 10.42 | 1.23 | .15 | 11.99 | .99 | .12 | 12.41 |
| Weight Kidney | 2.45 | .27 | 11.16 | 2.87 | .47 | 16.53 | 2.57 | .24 | 9.48 |
| Length Kidney | 2.43 | .13 | 5.22 | 2.49 | .15 | 5.98 | 2.46 | .13 | 5.25 |
| Weight Adrenal | .298 | .05 | 15.83 | .398 | .12 | 31.17 | .310 | .05 | 16.47 |
| Length Adrenal | 1.56 | .12 | 7.82 | 1.49 | .17 | 11.46 | 1.53 | .11 | 7.17 |
| Weight Spleen | .82 | .16 | 19.31 | .91 | .24 | 26.26 | .73 | .07 | 10.08 |
| Length Spleen | 3.14 | .26 | 8.21 | 3.74 | .45 | 12.12 | 2.99 | .18 | 5.85 |
| N = | 40 | | | 40 | | | 20 | | |

| | FAMILY 13♀ | | | FAMILY 2♂ | | | FAMILY 2♀ | | |
|---|---|---|---|---|---|---|---|---|---|
| | $\bar{V}$ | $\sigma$ | CV | $\bar{V}$ | $\sigma$ | CV | $\bar{V}$ | $\sigma$ | CV |
| Weight Body | 939.8 | 98.98 | 10.53 | 802.2 | 655.8 | 8.11 | 777.9 | 69.02 | 8.87 |
| Length Body | 30.97 | .61 | 1.96 | 29.99 | .62 | 2.07 | 29.48 | .54 | 1.83 |
| Weight Liver | 35.49 | 8.50 | 23.95 | 25.37 | 2.92 | 11.51 | 29.21 | 4.83 | 16.53 |
| Weight Lung | 7.44 | .88 | 11.80 | 5.21 | .97 | 18.67 | 5.18 | 1.22 | 23.55 |
| Weight Heart | 2.26 | .23 | 10.23 | 2.12 | .28 | 13.18 | 2.07 | .27 | 13.00 |
| Weight Thyroid | .074 | .924 | 32.69 | .061 | .013 | 21.31 | .058 | .007 | 11.21 |
| Length Thyroid | .95 | .08 | 8.36 | 1.27 | .15 | 11.93 | 1.20 | .14 | 11.81 |
| Weight Kidney | 2.33 | .15 | 6.25 | 2.94 | .56 | 18.90 | 2.79 | .38 | 13.52 |
| Length Kidney | 2.40 | .13 | 5.23 | 2.47 | .17 | 6.79 | 2.51 | .16 | 6.34 |
| Weight Adrenal | .284 | .041 | 14.43 | .402 | .13 | 34.57 | .394 | .11 | 27.95 |
| Length Adrenal | 1.58 | .14 | 8.78 | 1.39 | .14 | 10.26 | 1.59 | .17 | 10.69 |
| Weight Spleen | .93 | .16 | 16.84 | .78 | .12 | 15.29 | 1.03 | .27 | 26.35 |
| Length Spleen | 3.29 | .24 | 7.35 | 3.48 | .37 | 10.69 | 3.99 | .38 | 9.59 |
| N = | 20 | | | 20 | | | 20 | | |

* The weights are given in grams and the linear measurements in centimeters.

bilities that the means represent samples of the same population. The significance of the difference of each pair of means was obtained by calculating t according to the formula:

$$t = \frac{\Delta}{\sqrt{\frac{\Sigma(X_1^2 - (\Sigma X_1)^2/N_1) + \Sigma(X_2^2 - (\Sigma X_2)^2/N_2}{N_1 + N_2 - 2} \left(\frac{1}{N_1} + \frac{1}{N_2}\right)}}$$

in which X equals the weight or linear measurement, $\Delta$ equals the difference between the means, and N equals the number of individuals involved. The probability (P) was found in tables of the probability integral. (For a discussion of the formula used in the calculation of t see FISHER 1928.)

## DISCUSSION OF RESULTS

Table 2 shows that family 13 is significantly heavier than family 2. This difference, as well as differences in coat color, size of litter, frequency of litter, and vitality of young has been reported previously by WRIGHT (1922) and by WRIGHT and EATON (1929). While there is no question but what this difference is a genetic one, neither WRIGHT's nor the present data give any clue as to the exact number of gene loci involved. A few crosses suggest that there are at least several pairs of genes concerned.

Table 2 also shows that there is a sex difference with respect to body weight. The males of a given family are heavier than the females of the same family. This difference has also been reported previously by WRIGHT (1922). WRIGHT's data show the difference to be significant for both families but the difference in the present study is a significant one only for family 13. Why this should be true is not clear. It is possible that the explanation lies in the fact that the six males in a pen fought more than the six females and consequently added less weight. There is no question but what the males fought more. The sex difference with respect to body weight is undoubtedly due to a secondary effect of sex determining genes.

Family 13 also has a greater body length than family 2. This, of course, is what one would have expected on the basis of its greater body weight, although a reversal of this relationship could have existed. The positive correlation between body length and body weight suggests that the factors which increase body length are the same as those which are responsible for an increase in body weight. In other words, it would appear that there are general growth factors which increase both the length and the diameter or circumference of an animal.

There is also a sex difference with respect to body length. The males of both families have a greater body length than the females of their respective families. Evidently the sex determining genetic factors which second-

TABLE 2

*The probabilities that the means of the weights and linear measurements of the internal organs of two inbred families of guinea pigs are significantly different.*

| | STRAINS 13♂♀–♂♀ | | STRAINS 13♂–2♂ | | STRAINS 13♀–2♀ | | STRAINS 13♂–13♀ | | STRAINS 2♂–2♀ | |
|---|---|---|---|---|---|---|---|---|---|---|
| | t | p | t | p | t | p | t | p | t | p |
| Weight Body | 10.45 | .000 | 11.13 | .000 | 6.00 | .000 | 3.89 | .000 | 1.42 | .156 |
| Length Body | 11.36 | .000 | 9.94 | .000 | 8.19 | .000 | 4.49 | .000 | 2.76 | .006 |
| Weight Liver | 5.79 | .000 | 7.65 | .000 | 2.87 | .005 | .85* | .396 | 3.04* | .003 |
| Weight Lungs | 9.59 | .000 | 6.68 | .000 | 6.73 | .000 | 0.74* | .460 | 0.77 | .442 |
| Weight Heart | 3.97 | .000 | 3.15 | .000 | 2.40 | .017 | 1.80 | .072 | 0.55 | .583 |
| Weight Thyroid | 4.29 | .000 | 3.54 | .000 | 2.54 | .012 | 0.64 | .523 | 0.94 | .348 |
| Length Thyroid | 9.36* | .000 | 6.28* | .000 | 7.06* | .000 | 1.32 | .187 | 1.37 | .171 |
| Weight Kidney | 4.96* | .000 | 2.79* | .006 | 5.09* | .000 | 3.74 | .000 | 0.98 | .328 |
| Length Kidney | 1.89* | .059 | 0.40* | .690 | 2.20* | .028 | 1.16 | .247 | 0.76* | .447 |
| Weight Adrenal | 4.81* | .000 | 2.79* | .006 | 4.18* | .000 | 1.74 | .082 | 0.21 | .834 |
| Length Adrenal | 1.99 | .047 | 3.47 | .000 | 0.16* | .873 | 1.27* | .205 | 4.45* | .000 |
| Weight Spleen | 1.79* | .074 | 2.13* | .037 | 1.37* | .171 | 5.39* | .000 | 3.56* | .000 |
| Length Spleen | 7.24* | .000 | 5.33* | .000 | 6.96* | .000 | 4.48* | .000 | 4.30* | .000 |

* When no asterisk appears the group listed first in the heading has the heaviest or longest organ. When an asterisk appears the reverse is true.

arily cause an increase in the body weight of the males also cause an increase in their body length.

The liver, the lungs and the heart of family 13 are significantly heavier than the respective organs of family 2. EATON (1938) found the same relationship in other branches of the same two families. Since family 13 also has a greater body weight it appears that the size of each one of these organs is determined to a large extent by general body size factors. This, of course, does not preclude the possibility of variations due to genetic factors which affect them more specifically but the present data do not give any evidence of the effects of such factors.

There is a suggestion that the weight of the liver of the females is greater than that of the males of the same family but the difference is a significant one only for family 2.

Most surprising of all the results obtained are those pertaining to the thyroids. The thyroids of family 13 are definitely heavier than those of family 2. They are, however, much shorter. This seemingly contradictory statement is explained by the fact that the tissues of the thyroids of family

13 are compact, whereas, those of family 2 are loose and strung out. It would be of interest to find out if the thyroids of the two families differ in their secretory activity, but experiments to test this point have not been carried out.

The kidneys of family 2 are significantly heavier than those of family 13.

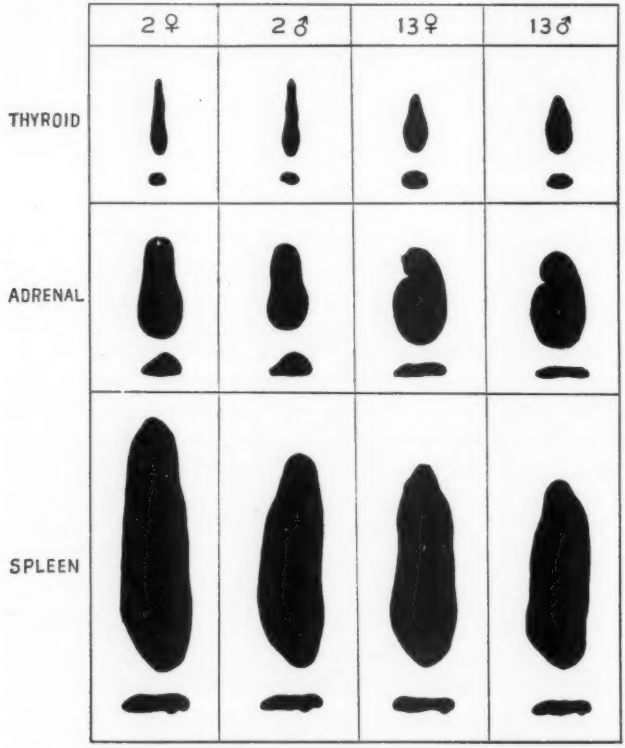


FIGURE 1.—Drawings showing differences in the size and shape of the thyroid, adrenal, and spleen of two highly inbred strains of guinea pigs (family 2 and family 13).

There is also a suggestion that they are longer but the difference is not quite great enough to be considered a significant one.

If no other differences had been found between family 2 and family 13 the two families could readily have been distinguished on the basis of the differences between their adrenals. The adrenals of family 2 are significantly heavier than those of family 13. The left adrenal of family 2 is thick and triangular in cross section as shown in figure 1, whereas, that of

family 13 is thin and flat. Furthermore, that of family 13 has a characteristic indentation on its mesial side. This peculiarity may occasionaly be present to a slight degree on the left adrenal of family 2 but it is never marked. There is a suggestion that the adrenals of the females of each family are longer than those of the males of the same family but the difference is a significant one only for family 2.

Most striking of all the differences observed are those of the spleen. The spleen of family 2 is long and narrow, whereas that of family 13 is short and broad. (See figure 1.) EATON (1938) found the same to be true in the branches of the two families which he studied. There is also a sex difference with respect to spleen size. That of the female is always longer and heavier than that of the male. This has been found to be true for all the strains of guinea pigs whose internal organs have been studied.

## SUMMARY AND GENERAL CONCLUSIONS

1. The liver, lungs, heart, thyroid, kidney, adrenal, and spleen of 20 males and 20 females of each of two highly inbred strains of guinea pigs are compared with respect to weight, linear measurement, and general shape.

2. The comparisons show that the liver, the lungs and the heart are significantly heavier in the one inbred strain (family 13) than in the other (family 2). Since family 13 also has a significantly greater body weight it seems probable that the size of each of these organs is determined to a large extent by general body size factors.

3. The thyroids, the adrenals and the spleen of the two families are different not only in size but in shape as well. This suggests that these organs may be affected by genetic factors which are quite independent of those which determine general body size.

4. The spleen of the female of each family is larger than that of the male of the same family. Evidently it is also affected secondarily by sex-determining factors.

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# THE INHERITANCE OF TAILLESSNESS (ANURY) IN THE HOUSE MOUSE. III. TAILLESSNESS IN THE BALANCED LETHAL LINE 19

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THREE lines of mice, each breeding true to complete absence of the tail, have been described in recent literature (DOBROVOLSKAIA-ZAWADSKAIA and KOBOZIEFF 1932, KOBOZIEFF 1935). Two of these, line A and line 29, have been shown (CHESLEY and DUNN 1936, DUNN 1937) to constitute balanced lethal lines, in which a dominant mutation *T* (short tail or Brachyury) which is lethal when homozygous is balanced by a recessive lethal which shows no crossing over with *T*. According to this interpretation line A is $Tt^0$, line 29 $Tt^1$, that is, each line contains the same Brachyury mutation *T*, and a recessive allele which is different in line A and line 29. Thus it comes about that each line produces only tailless progeny when bred *inter se*, but that crosses of line A tailless by line 29 tailless produce also normal tailed progeny $t^0t^1$ (DUNN 1937). Each of these lines was derived, according to KOBOZIEFF (1935) from an outcross of the short-tailed (Brachyuric $T+$) stock of ZAWADSKAIA to a different unrelated normal mouse.

The third line,[2] known as 19, was also derived from an outcross of the Brachyuric stock to an unrelated normal. This line was not studied extensively by KOBOZIEFF, although he was able to show that it bred true to taillessness, produced small litters, and sometimes gave Brachys when outcrossed to normal, thus indicating its heterozygous nature. CHESLEY and DUNN (1936) reported a few crosses of A tailless by 19 tailless; these consisted of tailless and normal progeny.

The experiments to be reported briefly herewith show that line 19 behaves in all respects like line 29 and that these two lines probably have the same genotype in respect to the *T* locus. The mutation in line 19 may thus represent a recurrence of the same mutation as that in line 29.

## EXPERIMENTAL DATA

Line 19 has been studied by the same methods as were employed with line A and line 29. The original stock of line 19 as received from DR. ZAWADSKAIA was outcrossed to our inbred Bagg albino (normal) stock and

[1] These experiments have been aided by the Fund for Research of Columbia University. DR. S. GLUECKSOHN-SCHOENHEIMER has given assistance and advice which is gratefully acknowledged.

[2] We are very grateful to DR. DOBROVOLSKAIA-ZAWADSKAIA for sending to us animals of this stock for study and to DR. WALTER LANDAUER and DR. BORIS EPHRUSSI for bringing this material to our laboratory.

by backcrossing to this stock brought into genetic similarity with the Brachy and tailless stocks.

Table 1 contains the principal results. In spite of many attempts, few successful matings of tailless by tailless occurred due to the high degree of

TABLE 1

*Results of matings of tailless mice of line 19* inter se *and with normals and Brachys.*

| EXP. | MATING | NORMAL ♀ | NORMAL ♂ | NORMAL ? | NORMAL TOTAL | BRACHY ♀ | BRACHY ♂ | BRACHY ? | BRACHY TOTAL | TAILLESS ♀ | TAILLESS ♂ | TAILLESS ? | TAILLESS TOTAL | GRAND TOTAL |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. | Tailless×tailless | | | | | | | | | 38 | 28 | 19 | 85 | 85 |
| 2. | Tailless ♀×normal ♂ | 13 | 12 | | 25 | 10 | 17 | | 27 | | | | | 52 |
| 3. | Normal ♀×tailless ♂ | 68 | 40 | 1 | 109 | 15 | 12 | | 27 | | | | | 136 |
| 4. | $F_1$ normal* ♀ (from 2)×Brachy ♂ | 54 | 43 | | 97 | 36 | 20 | | 56 | 24 | 22 | | 46 | 199 |
| 5. | $F_1$ normal† ♀ (from 3)×Brachy ♂ | 55 | 50 | | 105 | 26 | 34 | | 60 | 30 | 27 | | 57 | 222 |
| 6. | Normal ♀ $+t^z$×Brachy ♂ | 50 | 45 | | 95 | 16 | 19 | | 35 | 31 | 23 | | 54 | 184 |
| 7. | Total $+t^z$ ♀×Brachy ♂ | 159 | 138 | | 297 | 78 | 73 | | 151 | 85 | 72 | | 157 | 605 |
| 8. | Brachy ♀×$F_1$ ♂ (from 2)‡ | 66 | 64 | | 130 | 11 | 9 | | 20 | 62 | 59 | | 121 | 271 |
| 9. | Brachy ♀×$F_1$ ♂ (from 3)§ | 39 | 35 | | 74 | 6 | 12 | | 18 | 34 | 31 | | 65 | 157 |
| 10. | Brachy ♀×$+t^z$ ♂ | 24 | 28 | 5 | 57 | 2 | 4 | | 6 | 21 | 20 | 5 | 46 | 109 |
| 11. | Total | 129 | 127 | 5 | 261 | 19 | 25 | | 44 | 117 | 110 | 5 | 232 | 537 |
| 12. | Tailless ♀×Brachy ♂ | 4 | 9 | 2 | 15 | 5 | 4 | 2 | 11 | 2 | 8 | 4 | 14 | 40 |
| 13. | Brachy ♀×tailless ♂ | 66 | 61 | 2 | 129 | 11 | 5 | | 16 | 56 | 49 | 3 | 108 | 253 |

* 9 $F_1$'s tested
† 16 $F_1$'s tested
‡ 4 $F_1$'s tested
§ 4 $F_1$'s tested
} 33 proved to be $t^z+$.

infertility of tailless animals of both sexes. However, the 85 progeny from such matings when supplemented by the 34 obtained by KOBOZIEFF are sufficient to show that the line breeds true to taillessness. The litter size was about 4.0. Crosses of tailless female by normal male gave equal numbers of normal and Brachy offspring (exp. 2); and the $F_1$ normals (exps. 4 and 8) proved to be heterozygous for a recessive which combined with $T$ leads to the tailless phenotype. Denoting this recessive as $t^z$ we may assume that line 19 tailless is $Tt^z$. From crosses of tailless male ($Tt^z$) by normal ($++$) female we should thus expect equal numbers of Brachy ($T+$) and normal ($t^z+$). Actually there were obtained 109 normal: 27 Brachy, an excess of normals comparable to that obtained when line A or line 29 tailless males are crossed with normal females.

Normal $F_1$ females, and others of genotype $t^z+$, when tested by crossing with Brachy males, gave 297 normals, 151 Brachys and 157 tailless, clearly the 2:1:1 ratio expected (exp. 7). Normal brothers of these females, also $t^z+$, tested by Brachy females gave 261 normals, 44 Brachys and 232 tailless (exp. 11), a significant departure from the 2:1:1 ratio expected. The ratio disturbed was that between the Brachy ($T+$) and tailless ($Tt^z$) classes, which expresses the relative frequency of $+$ and $t^z$ gametes from the father.

A similar difference in reciprocal crosses was noted in crosses of tailless

by Brachy. Tailless female by Brachy male gave about equal numbers of normals, Brachys and tailless; whereas the reciprocal gave 129 normal, 16 Brachy and 108 tailless, again a marked excess of tailless relative to Brachy.

The breeding behavior of line 19 may be given the interpretation already applied to line A and line 29. Tailless animals contain the Brachy mutation $T$ together with a recessive $t^z$ which shows no crossing over with $T$. Females transmit the alleles at this locus normally, each allele ($T$ or $t^z$, or $+$ or $t^z$) going to half the eggs; males heterozygous for $t^z$, however, always transmit $t^z$ with higher frequency than its allele. These same peculiarities have been noted and discussed in connection with line A (CHESLEY and DUNN 1936) and line 29 (DUNN and GLUECKSOHN-SCHOENHEIMER 1939) and the present data add nothing of analytical importance. We have noted also that line 19 males, like line 29 males occasionally transmit the allele of $t^z$ in "clusters." Thus matings of line 19 tailless male 3299 by normal (Bagg albino) females gave 61 normals:22 Brachys. In one of these litters, however, there were 4 normals:9 Brachys, a significant departure from the total ratio, due to the association or "clustering" of the Brachys in this litter.

In line 19 as in line 29 (GLUECKSOHN-SCHOENHEIMER 1938) the homozygotes $t^z t^z$ die in the uterus before the time of implantation. DR. GLUECKSOHN-SCHOENHEIMER has kindly allowed me to quote unpublished data showing that four $+t^z$ females fertilized by $+t^z$ males and dissected on the eighth and ninth days of pregnancy contained a total of 45 implanted embryos of which 41 were alive and normal. Elimination of lethal embryos had occurred before implantation.

All three tailless lines studied, therefore, have their most unusual features in common: the balanced lethal condition, the sexual difference in segregation, and the "clustering" of certain types of gametes in the male. In the case of line A and line 29 this similarity is probably due to the presence of allelic mutations $t^0$ and $t^1$.

In the case of line 19, the allele of $T$ which is present is not $t^0$, for crosses of line A tailless by line 19 tailless have produced 33 tailless and 17 *normals*. The viability of the latter type is evidence that they do not contain the same lethal. Three normal-tailed females from this cross were tested by crossing with Brachy males. They produced 51 normal and 37 tailless and *no Brachys*. This conforms to the result expected if the $F_1$ normal females are $t^0 t^z$ and if no crossing over occurs between $t^0$ and $t^z$ (DUNN 1937) although the data are not sufficient to constitute a rigid test of this hypothesis.

On the other hand, reciprocal crosses of line 19 tailless by line 29 tailless have produced only tailless progeny, 92 in number. This would indicate either that the recessive lethal in line 19 is the same as in line 29, or that

line 19 contains an allele which is inviable in compound with the allele in line 29. In practice these two hypotheses cannot be distinguished. We may say, therefore, that 19 contains an allele of $T$ which is indistinguishable from the allele of line 29 and which we may designate $t^1$.

The three tailless lines appear to have arisen from three different outcrosses of the Brachy stock, and to this extent the mutations distinguishing them may have been of independent origin. The two lines (A and 29) certainly contain different mutant alleles, and thus two mutational steps are involved; line 19, however, may represent a recurrence of the mutation that produced line 29, or a similar mutation.

It is perhaps likely that three mutations occurred in normal stock from wild type gene to lethal allele: $+\rightarrow t^0$, $+\rightarrow t^1$, $+\rightarrow t^1$ and that each was brought into combination with $T$ when the Brachy stock was outcrossed to normal. If this is so then the several mutations have effects which are so unusual and so similar as to strengthen the assumption that they are in fact alternate conditions of the same locus.

## SUMMARY

1. A line of tailless mice obtained from DOBROVOLSKAIA-ZAWADSKAIA breeds true to taillessness, but segregates in outcrosses to normal or to short tail (Brachy). It contains two lethal mutations which show no crossing over and thus the stock constitutes a balanced lethal system.

2. Line 19 tailless resembles line 29 tailless (DUNN 1937) (a) in the possession of the same or a similar lethal allele $t^1$ of the Brachy mutation $T$. The genotype of line 19 is assumed to be $Tt^1$. Homozygotes $t^1t^1$ die before implantation, as in line 29. (b) In the transmission of $t^1$ by males with abnormally high frequency, although transmission by females is normal; (c) in the occurrence of "clusters" of gametes containing an allele of $t^1$ from fathers containing $t^1$.

3. Crosses of line 19 tailless by line A tailless produce both tailless and normal; crosses of 19 tailless by 29 tailless produce only tailless. It is concluded that the recessive allele $t^1$ in line 19 is different from that ($t^0$) in line A, but is either a recurrence or a similar mutation to that in line 29.

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# THE LINKAGE RELATIONS OF A NEW LETHAL GENE IN THE RAT (RATTUS NORVEGICUS)

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## INTRODUCTION

IN A RECENT paper (GRÜNEBERG 1938), a new lethal factor has been described in the rat which kills the homozygotes at any time between birth and about the fifth week of life, the majority of the animals dying during the first two weeks. The manifestations of this gene are noticeable in many parts of the body. The gene is therefore what used to be called "pleiotropic." A detailed analysis of these "pleiotropic" effects has shown, however, that all the manifold effects can be traced back to a primary anomaly of the cartilage. Many of the cartilages, notably those of the ribs and of the trachea, undergo an enormous increase in bulk. As a consequence, a very pronounced deformity of the thorax develops. The thoracic basket becomes a completely rigid carapace, and this structure is fixed in extreme inspiration. This leads to an inflation of the lungs (emphysema), and the animals eventually die from various consequences of the emphysema. For a detailed account of this somewhat complicated case of spurious pleiotropism, the reader is referred to the original paper.

In the meantime, it has been shown by H. B. FELL and H. GRÜNEBERG (1939) that the development of the cartilage anomaly does not depend on other disturbances in the body, such as an endocrine cause, but is a peculiarity of the cartilage cells themselves.

As was already pointed out in the first communication, the new lethal gene is linked to pink-eyed yellow ($p$) and therefore carried in the albino chromosome. More data about its linkage relations have now been collected and will be reported in this paper.

## THE SINGLE FACTOR RATIOS

In a total of 1782 animals from matings of two heterozygotes (tables 1–6), there were 1459 normal animals and 323 lethals. The percentage of lethals is therefore $18.13 \pm 1.03$ percent. This is significantly less that the expectation of 25 percent. The deficiency of lethals is almost certainly due to selective mortality during the first two or three days of life when no reliable classification is possible in the living animals. The pathological situation as described elsewhere makes it plausible that some of the lethals may die fairly soon after birth. On the other hand, the pathological mechanism of the anomaly would not point to a pre-natal elimination as a cause for the deficiency.

This is borne out by the following facts. In most cases, the number of living young was recorded within 24 hours after birth. Dividing this material in undepleted and depleted litters, the following figures are obtained.

| | NORMALS | LETHALS | TOTAL | PERCENTAGE OF LETHALS |
|---|---|---|---|---|
| Undepleted litters | 772 | 204 | 976 | 20.90 |
| Depleted litters | 580 | 105 | 685 | 15.33 |
| Total | 1352 | 309 | 1661 | 18.60 |

The percentage of lethals is therefore greater in undepleted litters, the difference being significant ($\chi^2 = 8.256$ for one degree of freedom; $P < 0.01$). It appears likely that the lethals were over-represented amongst the corpses, which were not included in the original records, and amongst the young already eaten by the mothers before the litters were discovered. The elimination of lethals, therefore, takes place largely, and probably entirely, after birth.

As is to be expected, the ratio of the marker genes linked to the lethal is secondarily disturbed, though to a lesser extent. In view of these disturbances of the ratios, the methods for the evaluation of these data have had to be somewhat modified. They are given in detail at the end of this paper in an appendix by J. B. S. HALDANE.

## LINKAGE OF THE LETHAL WITH PINK-EYED YELLOW

For the estimation of the crossover percentage between these genes, several sets of data are available. In table 1 are to be found the segregations of 13 families of the constitution *pl*/++ ♀ × *pl*/++ ♂ (coupling $F_2$ generation). The crossover percentage, which in this case is the mean of the recombination fractions of the two sexes, amounts to $20.58 \pm 2.20$ percent ($I = 2063.27$).

TABLE 1

*Linkage of p and l, coupling* $F_2$ (*pl*/++ ♀ × *pl*/++ ♂).

| MATING | + | *p* | *l* | *pl* | TOTAL |
|---|---|---|---|---|---|
| A | 30 | 5 | 6 | 7 | 48 |
| C | 43 | 8 | 6 | 9 | 66 |
| E | 25 | 1 | 1 | 4 | 31 |
| 58 | 25 | 4 | 0 | 2 | 31 |
| 60 | 37 | 6 | 6 | 10 | 59 |
| 68 | 25 | 5 | 1 | 3 | 34 |
| 76 | 27 | 8 | 3 | 4 | 42 |
| 78 | 18 | 2 | 0 | 3 | 23 |
| 79 | 56 | 8 | 1 | 6 | 71 |
| 82 | 28 | 1 | 7 | 7 | 43 |
| 117 | 19 | 0 | 3 | 3 | 25 |
| 119 | 14 | 3 | 0 | 1 | 18 |
| 120 | 8 | 0 | 0 | 1 | 9 |
| Total | 355 | 51 | 34 | 60 | 500 |

Black-eyed normal offspring obtained from some of the coupling $F_2$ matings (A, C, and E) were mated in pairs to produce an $F_3$. Two such matings showed segregation both for the lethal and for *p* (table 2). Such $F_3$ matings may theoretically be of three different kinds. Either both parents contain the two factors in coupling (C×C), or one contains the

TABLE 2

*Linkage of p and l, $F_3$*

| MATING | + | *p* | *l* | *pl* | TOTAL |
|---|---|---|---|---|---|
| 22 | 14 | 2 | 5 | 3 | 24 |
| 27 | 74 | 8 | 6 | 5 | 93 |
| Total | 88 | 10 | 11 | 8 | 117 |

genes in coupling and the other in repulsion (C×R), or finally both animals carry the factors in repulsion (R×R). The type of mating in every individual family can only be inferred from the offspring produced. In the case of table 2, it is fairly obvious that we are dealing with C×C matings. The recombination value for these two families is $q = 21.94 \pm 4.66$ percent ($I = 461.10$). In view of the uncertainty as regards the type of these $F_3$ matings, a correction of the crossover value is necessary (see appendix). In this case, the correction is trivial, being of the magnitude due to the mis-scoring of a single animal.

Another set of black-eyed normal rats produced by the coupling $F_2$ pairs was crossed to animals of the constitution *ppl*+. This type of cross is sometimes called a single-backcross. It allows the estimation of the recombination fractions of the two sexes separately, as it can be carried out reciprocally (*p*+*l*+ ♀ × *ppl*+ ♂, table 3, and *ppl*+ ♀ × *p*+*l*+ ♂, tables 4 and 5). In either case, the black-eyed animal may carry the genes in

TABLE 3

*Linkage of p and l, single backcrosses, coupling phase, female doubly heterozygous (pl/++ ♀ × pl/p+ ♂).*

| MATING | ♀ | ♂ | + | *p* | *l* | *pl* | TOTAL |
|---|---|---|---|---|---|---|---|
| 6 | C6 | A2 | 43 | 37 | 7 | 14 | 101 |
| 8 | C4 | C2 | 26 | 14 | 0 | 6 | 46 |
| 11 | E5 | E3 | 18 | 9 | 3 | 8 | 38 |
| 13 | A8 | A7 | 42 | 25 | 4 | 8 | 79 |
| 16 | C12 | C11 | 15 | 7 | 1 | 5 | 28 |
| 17 | C13 | C11 | 23 | 17 | 3 | 7 | 50 |
| 18 | C14 | C11 | 42 | 24 | 5 | 8 | 79 |
| 51 | A18 | A7 | 22 | 7 | 2 | 9 | 40 |
| 93 | A18 | A2 | 16 | 10 | 3 | 9 | 38 |
| 54 | A16 | A7 | 6 | 8 | 2 | 2 | 18 |
| 94 | A16 | A2 | 4 | 1 | 0 | 1 | 6 |
| Total | | | 257 | 159 | 30 | 77 | 523 |

coupling or repulsion. As judged by the progeny produced, 12 out of the 13 double heterozygotes listed in tables 3–5 appear to have carried the two

genes in coupling and one in repulsion. Again, a small correction for the uncertainty of this classification will be introduced in the appendix. Add-

TABLE 4

*Linkage of p and l, single backcrosses, coupling phase, male doubly heterozygous* (*pl*/*p*+ ♀ × *pl*/++ ♂).

| MATING | ♀ | ♂ | + | *p* | *l* | *pl* | TOTAL |
|---|---|---|---|---|---|---|---|
| 19 | C7 | C8 | 24 | 24 | 3 | 9 | 60 |
| 20 | A12 | C17 | 56 | 32 | 4 | 15 | 107 |
| 42 | C24 | E8 | 26 | 18 | 2 | 3 | 49 |
| Total | | | 106 | 74 | 9 | 27 | 216 |

ing to this the four individuals of the $F_3$, which were classified as coupling, the ratio is 16 C:1 R. C and R double heterozygotes are obtained from a coupling $F_2$ in the ratio $(1-q)^2:q^2$, where q is the recombination fraction. The observed ratio of 16:1 corresponds to q = 0.20, which is in good agreement with the estimate obtained from the coupling $F_2$.

In a coupling single-backcross with equal viability of all the four classes, the four phenotypes +, *p*, *l*, and *pl* are expected to occur in the ratio

$$2-q:1+q:q:1-q.$$

The product formula as given by IMMER (1930) leads to a satisfactory estimate of the crossover frequency even in those cases where this ratio is disturbed by a reduced viability of one of the genes. This is, however, not the case with IMMER'S formula for the variance of q, since those classes which give most of the information concerning q, namely the two lethal classes, are reduced in size. Hence it seems better to deal with normals and

TABLE 5

*Linkage of p and l, single backcross, repulsion phase, male doubly heterozygous* (*pl*/*p*+ ♀ × *p*+/+*l* ♂).

| MATING | ♀ | ♂ | + | *p* | *l* | *pl* | TOTAL |
|---|---|---|---|---|---|---|---|
| 21* | C15 | C16 | 8 | 29 | 3 | 1 | 41 |

* The ratio of black-eyed to pink-eyed is considerably distorted (11:30; $\chi^2 = 8.805$ for n = 1). The last litter (stillborn and therefore not classifiable for the lethal) contained five black-eyed and three pink-eyed young; if this litter is included, the ratio is 16:33 with $\chi^2 = 5.898$ for n = 1.

lethals separately. Two estimates for q are therefore obtained from each experiment. These values may then be combined, the weight given to each being proportional to the amount of information contained in the respective estimates.

By this treatment we obtain from the single backcross data for females

non-lethal classes $q' = 0.1466$; $I' = 195.75$

lethal classes $q'' = 0.2804$; $I'' = 530.32$

and the combined estimate is $q_{♀} = 0.2443 \pm 0.0371$ ($I = 726.07$). Similarly in the male, we obtain by combining the data of tables 4 and 5:

non-lethal classes $q' = 0.1370$; $I' = 103.39$
lethal classes $q'' = 0.2683$; $I'' = 208.85$

and the combined estimate is $q_{\sigma^{\prime}} = 0.2248 \pm 0.0566$ ($I = 312.24$). The recombination fraction is, therefore, higher in females than in males. The difference is not significant in the material presented here. But, as a corresponding difference in the same chromosome of the rat has been established beyond doubt by CASTLE and WACHTER (1924), I am confident that the sex difference observed here would have become significant had the sample been sufficiently increased.

It was next desired to combine the single-backcross data with the $F_2$ and $F_3$ data. The latter are average values for crossing over in the two sexes. Now it would be inaccurate simply to pool the *data* for the two sexes; such a value would be unduly influenced by the high crossover value of the females, which would contribute more than twice as much information as the males.

Hence it seems better to combine the crossover values for the two sexes by taking the arithmetical mean of the two *estimates* and ascribing to it the standard error $\pm\sqrt{m_1^2+m_2^2}$. This leads to a joint estimate for the two sexes from the single-backcross data of $q = 0.2346 \pm 0.0310$ ($I = 1038.31$).

We now have three estimates for the crossover value of $p-l$.

| | | |
|---|---|---|
| $F_2$ data | $q = 0.2058 \pm 0.0220$ | ($I = 2063.27$) |
| $F_3$ data | $q = 0.2194 \pm 0.0466$ | ($I = 461.10$) |
| Single-backcross data | $q = 0.2346 \pm 0.0310$ | ($I = 1038.31$) |
| | | $I = 3562.68$ |

As these estimates do not differ significantly, they may be combined by weighting according to the amount of information they contain respectively. The combined estimate is

$$q = 0.2160 \pm 0.0168 \quad (I = 3562.68).$$

This value, as was pointed out above, should be corrected for the fact that in the case of the $F_3$ and the single-backcrosses, the constitution of the double heterozygotes (whether C or R) was inferred from the offspring produced and consequently may have been faulty. The details of this correction are to be found in the appendix. It is very small and thus shows that the chance of erroneous classification of heterozygotes has been very slight in our case.

The crossover value obtained for the genes *p* and *l* has obviously to be compared with other linkage values established for this chromosome.

### LINKAGE OF THE LETHAL WITH ALBINISM

The crossover value between pink-eyed yellow and albinism (*c*) has

been determined with considerable accuracy by CASTLE and WACHTER (1924). A recalculation of their data leads to a crossover fraction of 0.2193 ±0.00583 in females and 0.1839±0.00397 in males. The joint estimate for the two sexes obtained from these data (arithmetical mean of the estimates) is 0.2016±0.00328. The distance between pink-eyed yellow and the lethal obtained in this paper appears to be somewhat greater, the difference being 0.0144±0.0171.

Under these circumstances, the lethal factor may be either fairly close to albinism or far removed. In the former case the order of the three genes may be either $l-c \cdots p$ or $c-l \cdots p$. If far removed from albinism, the order of the genes would be $c \cdots p \cdots l$.

If $c$ and $l$ are close together, their distance will probably not exceed 4.86 units, that is to say, it will presumably not be greater than the difference of the crossover values $p-l$ and $p-c$ plus twice its standard error. If, on the other hand, the order of genes is $c \cdots p \cdots l$, the map distance between $c$ and $l$ will probably not be smaller than 38.34 units; this is the sum of the $c-p$ and $p-l$ distances less twice its standard error. Owing to undetected double crossing over, the recombination percentage will be lower. If we assume that there is no interference, the expected recombination value will be about 3.7 units less than the map distance. Actually, the assumption of an absence of interference is probably wrong, as the existence of interference has been shown in the mouse in the region $sh_1 - c-p$ of the homologous chromosome (GRÜNEBERG 1935, 1936). Hence we are on the safe side if we assume that, for the order of genes $c-p-l$, the genes for albinism and the lethal should show a recombination percentage of at least 34. It had, therefore, to be determined whether the recombination percentage between albinism and the lethal is 4.86 units or less, or whether it is 34 units or more.

For this purpose, two females homozygous for black-eyed intense fur colcr and heterozygous for the lethal were outcrossed to a Wistar albino male. The $F_1$ animals thus produced were mated in pairs and thereby tested for heterozygosity for the lethal. The matings which segregated for albinism and the lethal are given in table 6.

The data about non-lethals yield practically no information about the crossover value and can be neglected. The two lethal classes lead to an estimate of q=0.1260 (for formula see appendix, table 7). The variance of this value has not been calculated as one class is represented by a single individual only, and the distribution will hence be far from normal.

We have, therefore, to calculate the probability that only one double recessive (or none) will be found in a sample of n (=63) individuals, if the crossover value is q (q=0.33, 0.34, 0.35 etc.). This probability is

$$P = (1 - q^2)^n + nq^2(1 - q^2)^{n-1}$$

TABLE 6

*Linkage of c and l, repulsion $F_2$ (c+/+l♀ × c+/+l♂).*

| MATING | + | *c* | *l* | *cl* | TOTAL |
|---|---|---|---|---|---|
| 98 | 43 | 11 | 17 | 0 | 71 |
| 108 | 22 | 7 | 5 | 0 | 34 |
| 110 | 23 | 11 | 11 | 0 | 45 |
| 110a | 43 | 14 | 6 | 1 | 64 |
| 122 | 21 | 11 | 8 | 0 | 40 |
| 129 | 29 | 15 | 3 | 0 | 47 |
| 133 | 18 | 16 | 7 | 0 | 41 |
| 140 | 19 | 8 | 3 | 0 | 30 |
| 150 | 5 | 6 | 2 | 0 | 13 |
| Total | 223 | 99 | 62 | 1 | 385 |

and yields the following values

| | | | |
|---|---|---|---|
| q = | .33 | .34 | .35 |
| P = | .0061 | .0040 | .0026 |

Hence even under the assumption that the expected crossover value is as low as .34, the chance of finding only one double recessive (or none) in a sample of 63 individuals is 1:250. It is, therefore, very unlikely that the order of genes is $c-p-l$.

On the other hand, the chance of finding at least one double recessive in a sample of n = 63 is

$$P = 1 - (1 - q^2)^n$$

and yields the following values

| | | | | | |
|---|---|---|---|---|---|
| q = | .05 | .04 | .03 | .02 | .01 |
| P = | .1459 | .0960 | .0551 | .0249 | .0063 |

Hence the observed ratio of 62 single recessives: 1 double recessive is compatible with the assumption that the distance between albinism and the lethal is about three to five units. We therefore conclude that the lethal gene is located close to albinism.

We have to consider next whether the order of the genes is $l-c \cdots p$ or $c-l \cdots p$. It was calculated above that the distance $p-l$ is $21.60 \pm 1.68$ units. It was further shown that the distance $c-l$ is unlikely to be less than three units. Now, if the lethal gene is located at least three units to the right of albinism, this would imply that the "true" distance between $p$ and $l$ is .1716 or less. This deviates from the observed value of .2160 $\pm$ .0168 by 2.64 times its standard error or more and is therefore very unlikely ($p \leqq .0041$). On the other hand, if the lethal is to the left of albinism, the range of "allowed" values starts rather less than once the standard error removed from the observed value. We therefore conclude that the lethal gene is located to the left of albinism, and that the order of genes is $l-c \cdots p$.

We have to consider next how many units the lethal gene is to the left

of albinism. For its localization, two sets of data are available. The experiments involving *l* and *p* put the lethal $1.44 \pm 1.71$ units to the left of albinism. The repulsion $F_2$ involving *l* and *c* puts the lethal 12.6 units to the left of *c*, but this value is only a very rough one. The locus of the lethal is likely to be somewhere between these values. Now, if we assume that it is ten units to the left of *c*, this estimate would agree very well with the repulsion $F_2$ data (*l* and *c*), but would be exceedingly unlikely in view of the experiments involving genes *l* and *p*. Similarly, if we assume the locus of *l* to be two units to the left of *c*, this would tally very well with the *l*−*p* data, but would be unlikely from the *l*−*c* experiment. Obviously, the most likely locus of the lethal is that which is equally likely from both bodies of data.

In practice, this locus is found most easily by a process of approximation. We assume provisionally that the lethal gene is located four units to the left of *c*. Then, for the *l*−*c* experiments, $P = 1-(1-q^2)^n$ for $n = 63$ and $q = .04$, which works out at .0960. For the *l*−*p* data, let $d_1$ be the estimated distance *l*−*p* and $d_2$ that of *c*−*p*, and $\sigma_{d_1}$ and $\sigma_{d_2}$ their standard errors respectively. Then

$$\frac{0.04 - (d_1 - d_2)}{\sqrt{\sigma_{d_1}^2 + \sigma_{d_2}^2}} = \frac{0.04 - (0.2160 - 0.2016)}{\sqrt{0.0168^2 + 0.00328^2}} = 1.497.$$

This corresponds, for one tail of the distribution, to $P = 0.0672$. The provisional value of 0.04 thus agrees better with the *l*−*c* data than with the *l*−*p* data and is, therefore, too great. We try next with a distance of 3.5 units and find for the *l*−*c* and *l*−*p* data values of P corresponding to 0.0743 and 0.1141 respectively. This time, we are grossly out in the opposite direction. Interpolating, we find that for a distance of 3.8 units the probabilities are respectively 0.0870 and 0.0868. We therefore adopt the distance of 3.8 units as the final value. In view of the size of the variances involved, greater accuracy seems unnecessary.

## DISCUSSION

The map of the albino chromosome of the rat now comprises five known genes, the greatest number for any mammalian chromosome so far. These five loci are

| | |
|---|---|
| *L*, *l* | normal, lethal |
| *C*, $c^r$, *c* | fully coloured, ruby-eyed, true albino |
| *R*, *r* | " " , red-eyed yellow |
| *P*, *p* | " " , pink-eyed yellow |
| *W*, *w* | normal, waltzer |

From the evidence presented by CASTLE and WACHTER (1924), KING and CASTLE (1937) and in this paper, the following map may be constructed

| *l* | *c* | *r* | *p* | *w* |
|---|---|---|---|---|
| 0 | 3.8 | 4.1 | 24.0 | 49 ± |

It is a remarkable fact that in the rat with a haploid chromosome number of 21, five out of 13 known genes should be found in one single chromosome. The albino chromosome of other rodents is similarly well stocked with known genes. In the mouse, it contains *shaker*$_1$, *c* and *p*, in the rabbit the genes for brown fur color, yellow fat and *c*, and in Peromyscus *c* and *p*. The genes for *c* and *p* are very probably homologous in all these species. But even so, the number of genes in the albino chromosome of the rodents remains remarkable and will require a special explanation. It appears inopportune to speculate on this problem now.

Early in the experiments described in this paper, the appearance of an agouti young in the offspring of two non-agouti parents was noticed (GRÜNEBERG 1937). This was explained as very probably due to a reverse mutation. It was pointed out, however, that the young in question might have appeared as the result of segregation, if there existed in the rat a hitherto unknown gene for dominant black epistatic over and linked to agouti. In that case, more agouti young should have occurred in the experiments described here. As that has not happened, it appears virtually certain that the hypothesis of an unknown dominant black can be discounted for the case under discussion.

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SUMMARY

A new lethal mutation in the rat causes abnormalities of the cartilage followed by emphysema of the lungs and death during the first six weeks of life. The percentage of lethals in $F_2$ matings is reduced below expectation by selective mortality after birth, but prior to the age at which a reliable classification is possible. Coupling $F_2$ and $F_3$ generations and single backcrosses involving *l* and *p*, and a repulsion $F_2$ generation involving *l* and *c* lead to a localization of the lethal 3.8 units to the left of albinism.

*Note Added in the Proof, July 8, 1939*

Since this paper was written, more material has been accumulated. The total of table 1 is now

| + | *p* | *l* | *pl* | Total |
|---|---|---|---|---|
| 408 | 56 | 37 | 64 | 565 |

The crossover fraction is .2026 ± .0207 ($I = 2342.45$) as compared with .2058 ± .0220 ($I = 2063.27$). The mean crossover value for the *l*–*p* section is thereby slightly lowered from .2160 ± .0168 ($I = 3562.68$) to .2133 ± .0161 ($I = 3841.86$).

The total of table 6 is now

| + | *c* | *l* | *cl* | Total |
|---|---|---|---|---|
| 325 | 161 | 87 | 1 | 574 |

lowering the crossover value for the *l*–*c* section from .1260 to .1066. The chance that the order of genes is *c*–*p*–*l*, even assuming a recombination value as low as .33, is now only .00046 (formerly .0061). The most likely locus of the lethal is now 3.3 units to the left of *c*, as compared with 3.8 units before.

## *APPENDIX*

### *The analysis of linkage data where the type of linkage is uncertain*

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DR. GRÜNEBERG's data raise a problem which is likely to occur in a number of other cases, particularly where certain genotypes are lethal or sterile, so that the composition of double heterozygotes is uncertain. Whilst it turns out that in the particular case in question the corrections to be made are quite trivial, this will not always be so; and even in DR. GRÜNEBERG'S case it has to be shown that the uncertainty with regard to parental genotypes is unimportant.

Two types of $PpLl \times ppLl$ (or reciprocal) matings are possible, with the following expectations. Since there is a shortage of lethals, the proportions given are those of dark-eyed to pink-eyed among normals and lethals respectively.

| | | + | *p* | *l* *pl* |
|---|---|---|---|---|
| $\frac{++}{pl} \times \frac{p+}{pl}$ | (C) | $\frac{2-q}{3}:\frac{1+q}{3}:$ | | $q:1-q$ |
| $\frac{+l}{p+} \times \frac{p+}{pl}$ | (R) | $\frac{1+q}{3}:\frac{2-q}{3}:$ | | $1-q:q$ |

Here q is the crossover frequency, supposed equal in both sexes.

Similarly three types of $PpLl \times PpLl$ matings are possible, with the following expectations:

| | | + | *p* | *l* *pl* |
|---|---|---|---|---|
| $\frac{++}{pl} \times \frac{++}{pl}$ | (CC) | $\frac{3-2q+q^2}{3}:\frac{2q-q^2}{3}$ | : | $2q-q^2:1-2q+q^2$ |
| $\frac{++}{pl} \times \frac{+l}{p+}$ | (CR) | $\frac{2+q-q^2}{3}:\frac{1-q+q^2}{3}:$ | | $1-q+q^2:q-q^2$ |
| $\frac{+l}{p+} \times \frac{+l}{p+}$ | (RR) | $\frac{2+q^2}{3}:\frac{1-q^2}{3}$ | : | $1-q^2:q^2$ |

Some families can be assigned with certainty to one type. Others are doubtful. In the latter case the following procedure is adopted. We take the linkage value established on the basis of the crude data, giving each family its most probable origin, and calculate, first the probability *a priori* that a mating was of a given type, and secondly the probability that a mating of that type could have given the observed family. This yields a weighting factor.

In each case we consider a group of families consisting of:

$$\alpha+, \quad \beta p, \quad \gamma l, \quad \delta pl.$$

As the expected ratio of lethals to non-lethals is unknown, we must base our estimates on the lethals and non-lethals separately, and pool them, weighted according to the amount of information. If $q_r$ and $I_r$ are the estimated crossover values, and amounts of information concerning them, our final estimate is $\Sigma q_r I_r / \Sigma I_r$. Hence the values of $q_r I_r$ are needed.

TABLE 7

| GROUP OF SUB-FAMILIES | q | I | qI |
|---|---|---|---|
| C non-lethals | $\frac{2\beta-\alpha}{\alpha+\beta}$ | $\frac{(\alpha+\beta)^3}{9\alpha\beta}$ | $\frac{(\alpha+\beta)^2(2\beta-\alpha)}{9\alpha\beta}$ |
| C lethals | $\frac{\gamma}{\gamma+\delta}$ | $\frac{(\gamma+\delta)^3}{\gamma\delta}$ | $\frac{(\gamma+\delta)^2}{\delta}$ |
| R non-lethals | $\frac{2\alpha-\beta}{\alpha+\beta}$ | $\frac{(\alpha+\beta)^3}{9\alpha\beta}$ | $\frac{(\alpha+\beta)^2(2\alpha-\beta)}{9\alpha\beta}$ |
| R lethals | $\frac{\delta}{\gamma+\delta}$ | $\frac{(\gamma+\delta)^3}{\gamma\delta}$ | $\frac{(\gamma+\delta)^2}{\gamma}$ |
| CC non-lethals | $1-\sqrt{\frac{\alpha-2\beta}{\alpha+\beta}}$ | $\frac{4(\alpha+\beta)^2(\alpha-2\beta)}{9\alpha\beta}$ | $1-\frac{4\sqrt{(\alpha+\beta)^2(\alpha-2\beta)^3}}{9\alpha\beta}$ |
| CC lethals | $1-\sqrt{\frac{\delta}{\gamma+\delta}}$ | $\frac{4(\gamma+\delta)^2}{\gamma}$ | $1-\frac{4\sqrt{(\gamma+\delta)^3\delta}}{\gamma}$ |
| CR non-lethals | $\frac{1}{2}-\frac{1}{2}\sqrt{\frac{(3(3\beta-\alpha)}{\alpha+\beta}}$ | $\frac{(\alpha+\beta)^2(3\beta-\alpha)}{3\alpha\beta}$ | $\frac{1}{2}I-\frac{1}{2\alpha\beta}\sqrt{\frac{(\alpha+\beta)^3(3\beta-\alpha)^3}{3}}$ |
| CR lethals | $\frac{1}{2}-\frac{1}{2}\sqrt{\frac{\gamma-3\delta}{\gamma+\delta}}$ | $\frac{(\gamma+\delta)^2(\gamma-3\delta)}{\gamma\delta}$ | $\frac{1}{2}I-\frac{\sqrt{(\gamma+\delta)^3(\gamma-3\delta)^3}}{2\gamma\delta}$ |
| RR non-lethals | $\sqrt{\frac{\alpha-2\beta}{\alpha+\beta}}$ | $\frac{4(\alpha+\beta)^2(\alpha-2\beta)}{9\alpha\beta}$ | $\frac{4\sqrt{(\alpha+\beta)^3(\alpha-2\beta)^3}}{9\alpha\beta}$ |
| RR lethals | $\sqrt{\frac{\delta}{\gamma+\delta}}$ | $\frac{4(\gamma+\delta)^2}{\gamma}$ | $\frac{4\sqrt{(\gamma+\delta)^3\delta}}{\gamma}$ |

An example of the calculation is as follows. Consider the CR non-lethals. Let $x = q - q^2$. The expected values of $\alpha$ and $\beta$ are

$$\frac{2+x}{3}(\alpha+\beta) \text{ and } \frac{1-x}{3}(\alpha+\beta). \text{ Hence } x = \frac{\alpha-2\beta}{\alpha+\beta},$$

$$L = \alpha \log (2 + x) + \beta \log (1 - x),$$

$$I_x = -\frac{d^2L}{dx^2} = \frac{\alpha}{(2+x)^2} + \frac{\beta}{(1-x)^2} = \frac{(\alpha+\beta)^3}{9\alpha\beta},$$

$$I_q = \left(\frac{dx}{dq}\right)^2 I_x = (1 - 4x)I_x = \frac{(\alpha+\beta)^2(3\beta-\alpha)}{3\alpha\beta}$$

$$q = \tfrac{1}{2}(1 - \sqrt{1-4x}) = \tfrac{1}{2} - \frac{3^{1/2}(3\beta-\alpha)^{1/2}}{(\alpha+\beta)^{1/2}}.$$

Hence $$qI_q = \tfrac{1}{2}I_q - \frac{(\alpha+\beta)^{3/2}(3\beta-\alpha)^{3/2}}{2\cdot 3^{1/2}:\alpha\beta}.$$

Substituting the observed values of $\alpha$, $\beta$, $\gamma$, $\delta$, we have the results of table 8, assuming that all rats have the genotype most obviously attributed to them. The weighted mean value of q is $.2213 \pm .0169$. We could, of course, estimate the value of q in the case of CC, CR, and RR matings from the value of $\alpha\delta/\beta\gamma$. This gives substantially the same result. Thus the CC figures of table 8 give $q = .215$, and .212 by the method of this paper. The method here given has the merit of showing that, where numbers are large, the lethals and non-lethals give much the same values of q.

TABLE 8

| SUB-FAMILIES | TABLES | $\alpha, \beta$; OR $\gamma, \delta$ | q | I | qI |
|---|---|---|---|---|---|
| C non-lethals | 3 and 4 | 363,233 | .2181 | 278.13 | 60.66 |
| C lethals | 3 and 4 | 39,104 | .2727 | 707.21 | 192.63 |
| R non-lethals | 5 | 8,29 | −.3514 | 24.26 | −8.52 |
| R lethals | 5 | 3,1 | .2500 | 21.33 | 5.33 |
| CC non-lethals | 1 and 2 | 443,61 | .2023 | 1341.07 | 271.29 |
| CC lethals | 1 and 2 | 45,68 | .2243 | 1135.02 | 254.54 |
| | | | .2213 | 3607.02 | 775.93 |

We must now ask how far this will be changed if we allow for the fact that the genotypes of some parents may have been incorrectly given. Most of the information is derived from the $F_2$, where there is no uncertainty, and which gives $q = .2058$. In the case of a *PpLl* rat belonging to $F_2$, the *a priori* probability that it should be $++/pl$ is $(1-q)^2/(1-q)^2+q^2$, or, taking q as .2213, .9253. The probability that it should be $+l/+p$ is .07473. We have next to determine the *a posteriori* probability. In a back-cross the probabilities that $++/pl$ and $+l/p+$ rats respectively should give $\alpha+$, $\beta p$, $\gamma l$, $\delta pl$ offspring when crossed with $p+/pl$ are respectively:

$$\frac{(\alpha+\beta)!}{\alpha!\beta!}\left(\frac{2-q}{3}\right)^{\alpha}\left(\frac{1+q}{3}\right)^{\beta}\frac{(\gamma+\delta)!}{\gamma!\delta!}q^{\gamma}(1-q)^{\delta},$$

and

$$\frac{(\alpha+\beta)!}{\alpha!\beta!}\left(\frac{1+q}{3}\right)^{\alpha}\left(\frac{2-q}{3}\right)^{\beta}\frac{(\gamma+\delta)!}{\gamma!\delta!}(1-q)^{\gamma}q^{\delta},$$

whose quotient is

$$\left(\frac{2-q}{1+q}\right)^{\alpha-\beta}\left(\frac{1-q}{q}\right)^{\delta-\gamma}.$$

If then P is the probability that an $F_2$ rat which has produced such a family is $++/pl$,

$$\frac{P}{1-P}=\left(\frac{2-q}{1+q}\right)^{\alpha-\beta}\left(\frac{1-q}{q}\right)^{2+\delta-\gamma}$$
$$=1\cdot4564^{\alpha-\beta}\,3\cdot5188^{2+\delta-\gamma}.$$

$1-P$ is less than .001 except in the following families: (54+94), P = .98449; 42, P = .99887. In family 21, P = .00008.

Similarly in the case of $F_3$ matings the *a priori* probabilities of CC, CR, and RR as $(1-q)^4:2q^2(1-q)^2:q^4$. Hence the relative probabilities that a given family is CC, CR, and RR are:

$$\text{CC}\quad(3-2q+q^2)^{\alpha}(2q-q^2)^{\beta+\gamma}(1-q)^{2\delta+4}=\lambda P_1$$
$$\text{CR}\quad 2(2+q-q^2)^{\alpha}(1-q+q^2)^{\beta+\gamma}(q-q^2)^{\delta+2}=\lambda P_2$$
$$\text{RR}\quad(2-q^2)^{\alpha}(1-q^2)^{\beta+\gamma}q^{2\delta+4}=\lambda P_3$$

where $P_1$, $P_2$, $P_3$ are the actual probabilities, and $\lambda$ a constant. Hence:

$$P_1/P_3=1\cdot2729^{\alpha}\times\cdot41285^{\beta+4}\times3\cdot5188^{2\delta+4}$$
$$P_2/P_3=2\times1\cdot0602^{\alpha}\times\cdot8703^{\beta+\gamma}\times3\cdot5188^{\delta+2}.$$

In family 22, $\alpha=14$, $\beta+\gamma=7$, $\delta=3$, so $P_1/P_3=17442$, $P_2/P_3=924.95$, so $P_1=.9496$, $P_2=.05035$, $P_3=.000054$. In family 27, $P_1=1.0000$, $P_2=.0000$, $P_3=.0000$.

Thus the probability of incorrect assignment nowhere exceeds 5.04 percent, and the value of q is clearly not altered appreciably. However, the corrections will be made so that the method may be applied in future cases.

A correction of $\alpha=.184$, $\beta=.158$, $\gamma=.0331$, $\delta=.0498$ must be subtracted from the C and added to the R families. A correction of $\alpha=.705$, $\beta=.101$, $\gamma=.252$, $\delta=.151$ must be subtracted from the CC families, and put as a CR family. The total correction is thus of the magnitude due to the misscoring of a single rat.

At first sight the correction cannot be made, since the value of q deduced from the CR "family" is imaginary. However, such small corrections can be made by answering the following question. "If there were already a large number of families of a given type, giving the standard value of q, what corrections to I and qI would be made as a result of the correction

TABLE 9

*Formulae for small corrections.*

| GROUP OF SUB-FAMILIES | ΔI | ΔqI |
|---|---|---|
| C non-lethals | $\frac{3[(1-q)^2\alpha'+(2q-q^2)\beta']}{(2-q)^2(1+q)^2}$ | $\frac{-2(1+q)(1-q+q^2)\alpha'+(2-q)(2+q+2q^2)\beta'}{(2-q)^2(1+q)^2}$ |
| C lethals | $\frac{(1--q)(3q-1)\gamma'+q(2-3q)\delta'}{q^2(1-q)^2}$ | $\frac{2(1-q)\gamma'+(1-2q)\delta'}{(1-q)^2}$ |
| R non-lethals | $\frac{3[(2q-q^2)\alpha'+(1-q^2)\beta^2]}{(2-q)^2(1+q)^2}$ | $\frac{(2-q)(2+q+2q^2)\alpha'-2(1+q)(1-q+q^2)\beta'}{(2-q)^2(1+q)^2}$ |
| R lethals | $\frac{q(2-3q)\gamma'+(1-q)(3q-1)\delta'}{q^2(1-q)^2}$ | $\frac{(1-2q)\gamma'+2(1-q)\delta'}{(1-q)^2}$ |
| CC non-lethals, $x=(1-q)^2$ | $\frac{4[2(1+x)(1+x+x^2)\alpha'-(2+x)(2-x+2x^2)\beta']}{(2+x)^2(1-x)^2}$ | $\frac{\beta'}{1-x}-\frac{\alpha'}{2+x}+\frac{6q[(1-x)(2+x+x^2)\alpha'-(2+x)(2-x+x^2)\beta']}{(2+x)^2(1-x)^2}$ |
| CC lethals, $x=(1-q)^2$ | $\frac{4[(1-2x)\gamma'+2(1-x)\delta']}{(1-x)^2}$ | $\frac{2\gamma'}{1-x}-\frac{2\delta'}{x}+\frac{2q[x(1-3x)\gamma'+(1-x)(1+3x)\delta']}{x(1-x)^2}$ |
| CR non-lethals, $y=q-q^2$ | $\frac{-(1-y)(5+5y+8y^2)\alpha'+(2+y)(8-7y+8y^2)\beta'}{(2+y)(1-y)^2}$ | $\frac{-\alpha'}{2+y}+\frac{\beta'}{1-y}+\frac{3q[-(1-y)(3+y+2y^2)\alpha'+(2+y)(4-3y+2y^2)\beta']}{(2+y)^2(1-y)^2}$ |
| CC lethals, $y=q-q^2$ | $\frac{y(2-7y+8y^2)\gamma'-(1-y)(1-3y+8y^2)\delta'}{y^2(1-y)^2}$ | $\frac{-\gamma'}{1-y}+\frac{\delta'}{y}+\frac{q[y(2-5y+6y^2)\gamma'-(1-y)(1-y+y^2)\delta']}{y^2(1-y)^2}$ |

to the values of $\alpha$, $\beta$, $\gamma$, and $\delta$?" This correction is independent of the original number, provided it is large.

Consider a group of C families. If there were s non-lethals, the original values of $\alpha$ and $\beta$ being $\frac{1}{3}(2-q)s$ and $\frac{1}{3}(1+q)s$, and the corrected values $\frac{1}{3}(2-q)s+\alpha'$ and $\frac{1}{3}(1-q)s+\beta'$, then if $I+\Delta I$ is the corrected value of I, we find

$$I = \frac{s}{(1+q)(2-q)}, \qquad I + \Delta I = \frac{(s+\alpha'+\beta')^3}{[(2-q)s+3\alpha'][(1+q)s+3\beta']}.$$

Hence

$$\Delta I = \frac{3[(1-q)^2\alpha' + (2q-q^2)\beta']}{(2-q)^2(1+q)^2} \text{ approximately.}$$

Similarly

$$qI = \frac{qs}{(1+q)(2-q)}, \quad qI + \Delta qI = \frac{(s+\alpha'+\beta')^2(qs-\alpha'+2\beta')}{[(2-q)s+3\alpha'][(1+q)s+3\beta']}$$

Hence

$$\Delta qI = \frac{-2(1+q)(1-q+q^2)\alpha' + (2-q)(2+q+2q^2)\beta'}{(2-q)^2(1+q)}.$$

The formulae needed are given in table 9. Applying them to the corrections to $\alpha$, $\beta$, $\gamma$, and $\delta$ we find that I must be diminished by 19.78, and qI by 1.86. Thus our final value of q is $.2220 \pm .0169$.

As the value of q is probably different in the two sexes, this value is probably too high, since more weight was given to doubly heterozygous females than males. DR. GRÜNEBERG's value of .2160 is somewhat more plausible, but in view of the standard error of 1.7 percent no decision can be reached.

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